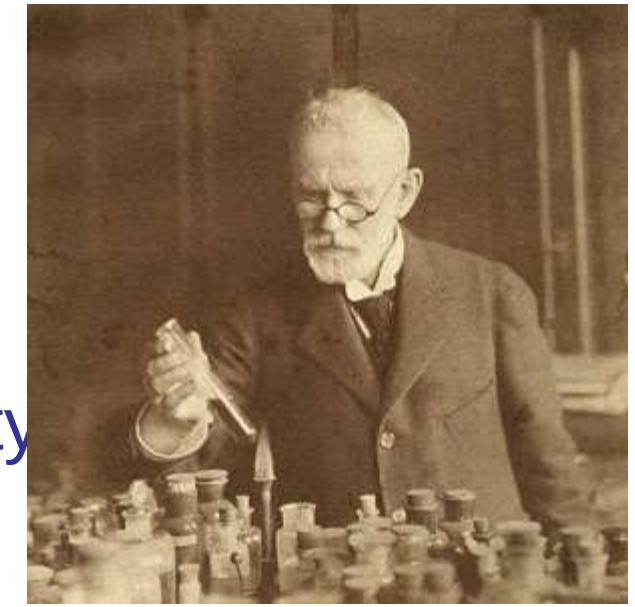
Antimicrobial drugs I.

Principles of the antibacterial chemotherapy
Modes of action and interactions

Dora Szabo

Institute of Medical Microbiology

History



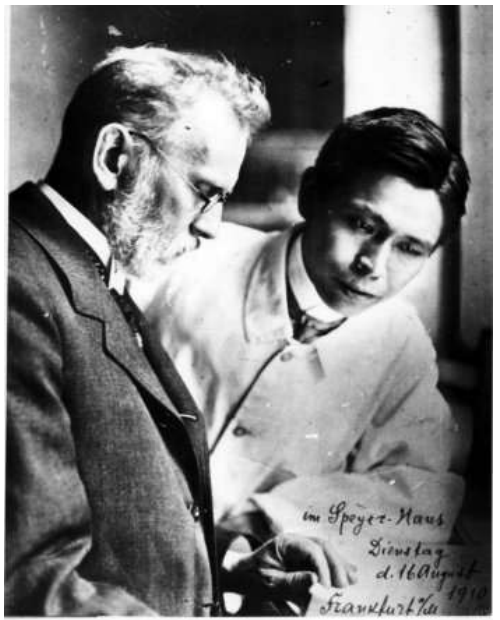
Paul Ehrlich

"Magic Bullet"

- Chemicals with selective toxicity
- together with Sahachiro Hata have arsenic and aniline dyes derivatives sought
  606 derivative, Salvarsan was effective against syphilis
- ORIGIN: Selective Stains

DRUG: Arsphenamine (1910)

"606" Salvarsan



History

Gerhard Domagk

- ✓ **Drugs are changed in the body**

ORIGIN: **Prontosil**

(Only active *in vivo*)

DRUG: **Sulfanilamide** **(1935)**

NOBEL: **1939**

History

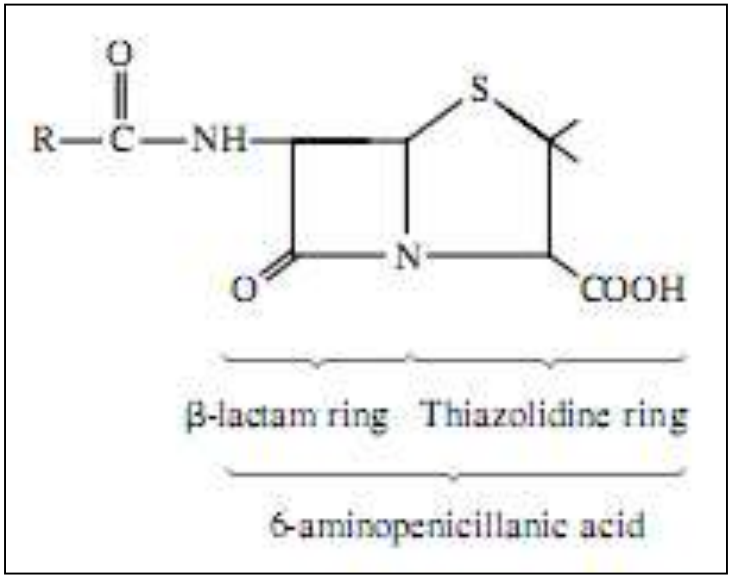
Alexander Fleming

- Microbes make antibiotics

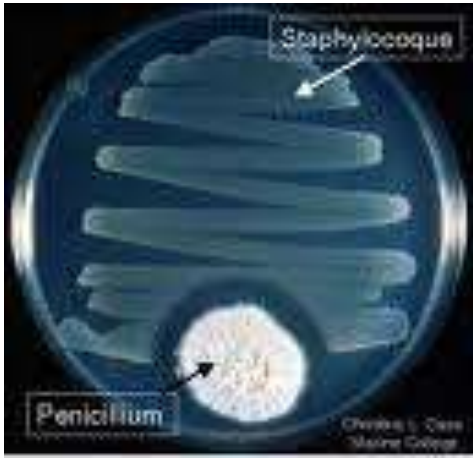
ORIGIN:moldy culture plate

DRUG: Penicillin (1928)

NOBEL: 1945



Penicillin



- 1945 Nobel-price:

  Fleming Florey Chain

History

Selman Abraham Waksman

- Soil Streptomyces make antibiotics
- comes up with definition of antibiotic
- Streptomycin the first antituberculoticum

ORIGIN: Penicillin development

DRUG: Streptomycin (1943)

NOBEL: 1952

Antimicrobial effect

- **Extracorporal**
  - Desinfection
  - Sterilization
- **Intracorporal**
  - Chemotherapeutic agents
  - Antibiotica

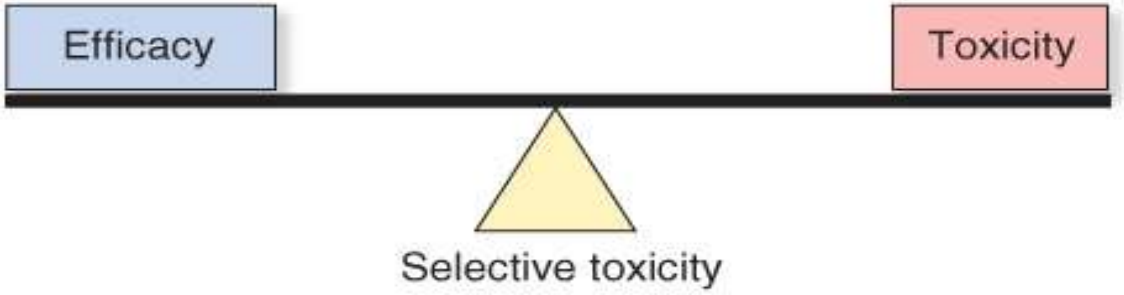
Antimicrobial drugs:

Chemotherapeutic agent- drug synthesised chemically

Antibiotic – natural agent synthesised by a bacterial or fungal strain

Selective Toxicity

- Cause greater harm to microorganisms than to host
- Chemotherapeutic index= lowest dose toxic to patient divided by dose typically used for therapy



$$\text{Chemotherapeutic index} = \frac{\text{Toxic dose}}{\text{Therapeutic dose}}$$

Chemotherapeutic index= :

$$Ki = \frac{\text{dosis tolerata maxima}}{\text{dosis curativa minima}}$$

The higher the index, the more effective chemotherapeutic agent (DTM/DCM)

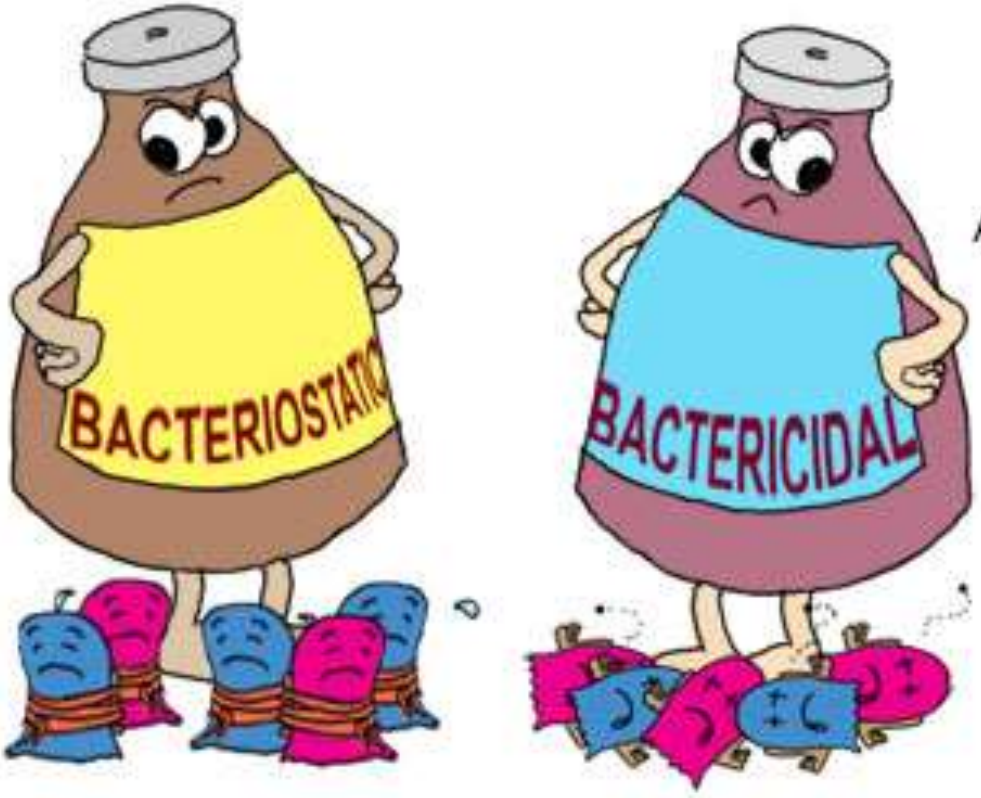
Antibiotics can be either

- **Broad Spectrum**
  - **Kill a wide range of bacteria e.g. Penicillin**

- **Narrow Spectrum**
  - **Kill a specific type or group of bacteria e.g. Isoniazid**

- **Bacteriostatic**, i.e. those that act primarily by arresting bacterial multiplication,
- **Bactericidal**, i.e. those which act primarily by killing bacteria

EXAMPLES:
Chloramphenicol
Erythromycin
Clindamycin
Sulfonamides
Trimethoprim
Tetracyclines



EXAMPLES:
Aminoglycosides
Beta-lactams
Vancomycin
Quinolones
Rifampin
Metronidazole

- **MIC (minimal inhibitory concentration)**
  ⇒ the lowest concentration (highest dilution) of the drug that has an inhibitory (bacteriostatic) effect.

  - Tube dilution
  - Microdilution
  - Agardilution
  - E-test

- **MBC (minimal bactericidal concentration)**
  ⇒ the lowest concentration (highest dilution) that has a killing (bactericidal) effect

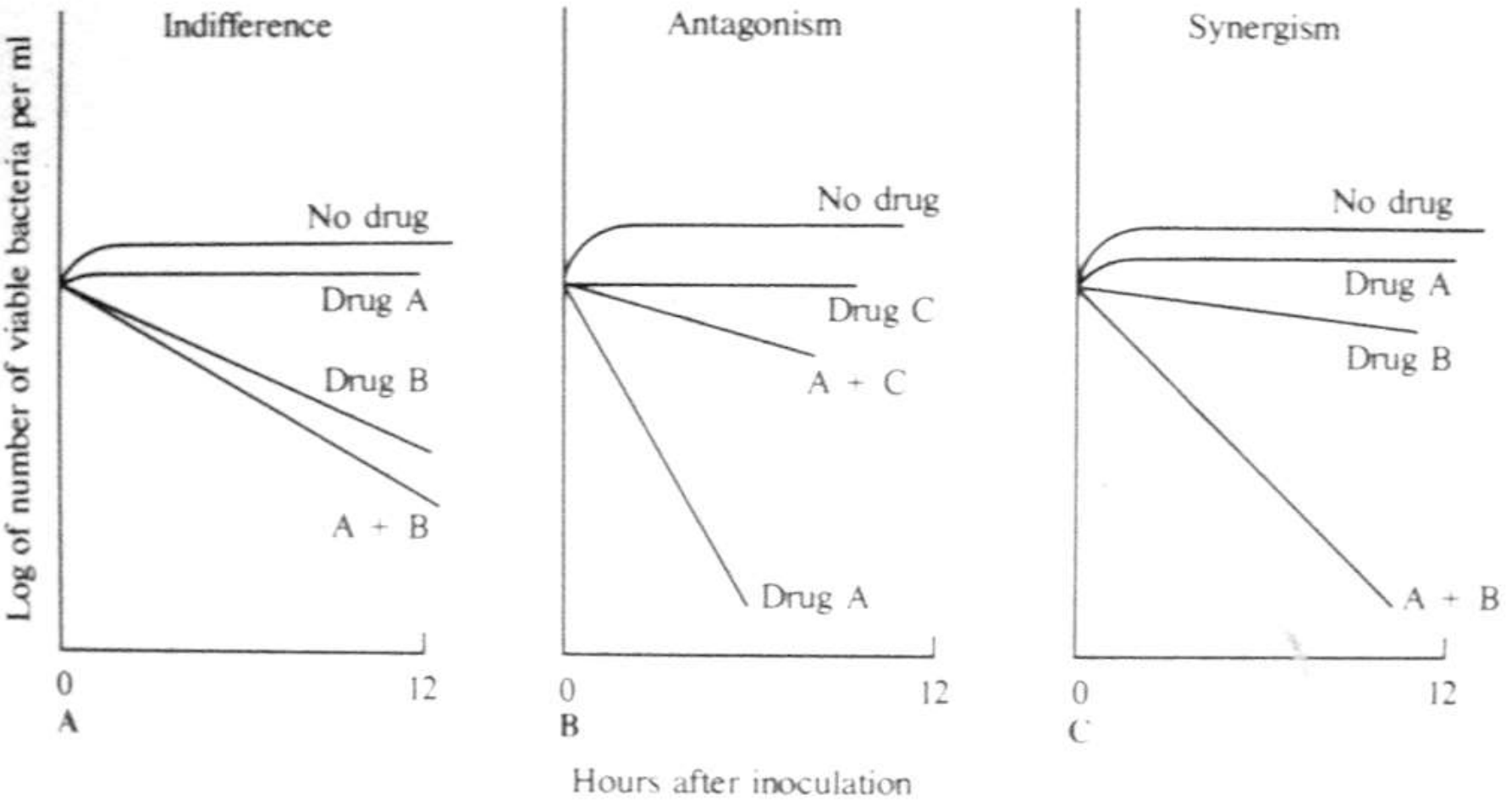
The Ideal Drug*

1. Selective toxicity: against target pathogen but not against host
   - **LD_{50}** (high) vs. **MIC** and/or **MBC** (low)
2. Bactericidal vs. bacteriostatic
3. Favorable pharmacokinetics: reach target site in body with effective concentration
4. Spectrum of activity: broad vs. narrow
5. Lack of "side effects"
   - Therapeutic index: effective to toxic dose ratio
6. Little resistance development

* **There is no perfect drug.**

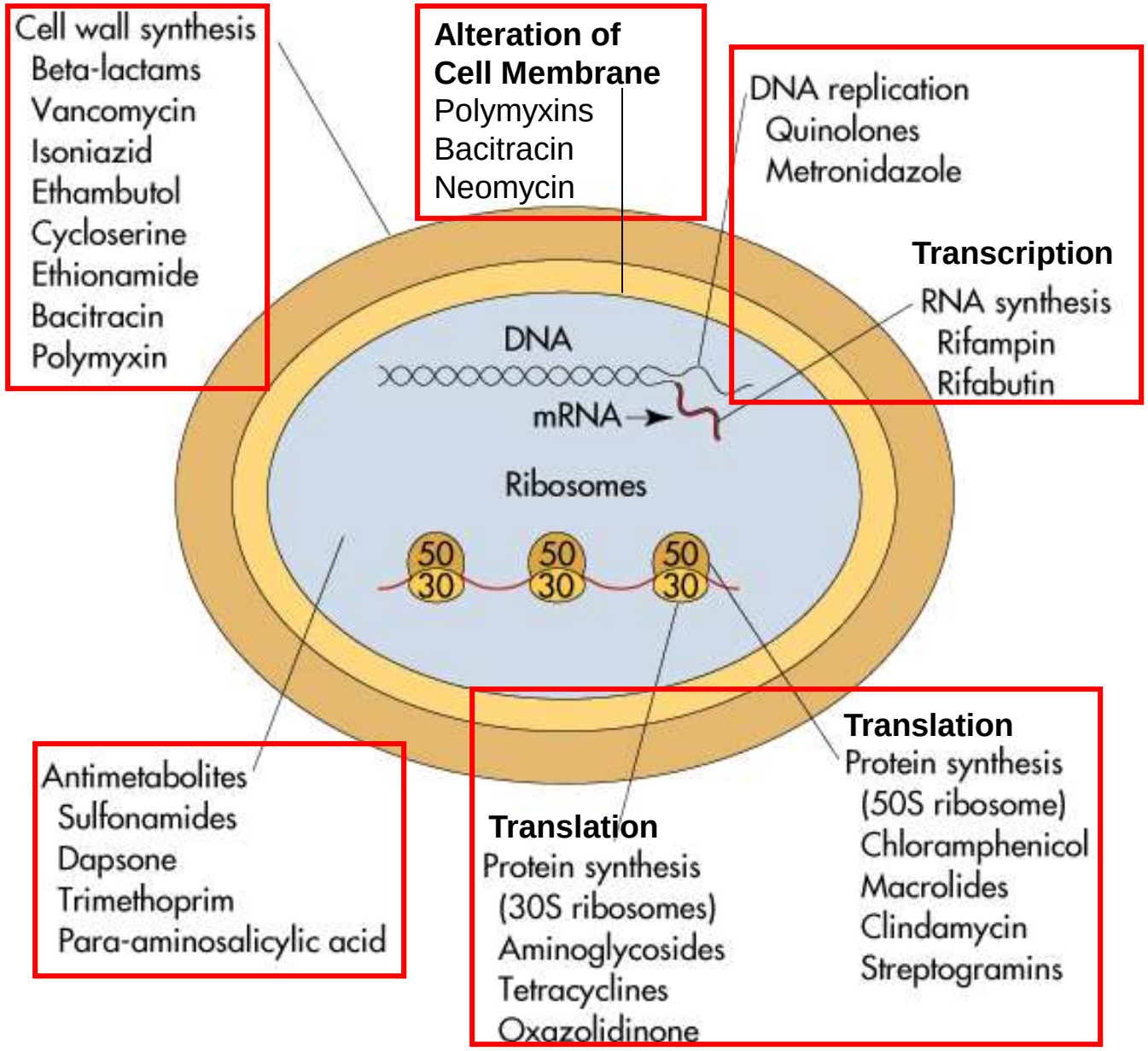
Side effects of chemotherapy

- Allergic response
- Toxic effects
- Disbacteriosis
- Inhibition of immune system
- Embryotoxic action
- Formation of the drug resistance



Types of combined actions of antimicrobial

Antibiotic Mechanisms of Action

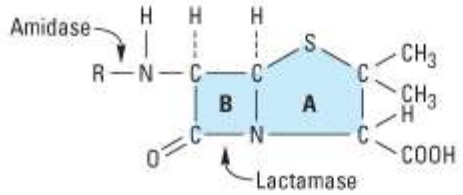


Beta-lactam antibiotics

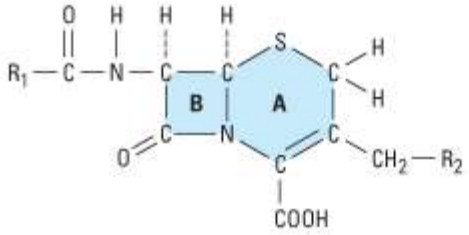
BETA-LACTAM ANTIBIOTICS
(inhibitors of cell wall synthesis)

Their structure contains a beta-lactam ring.
The major subdivisions are:

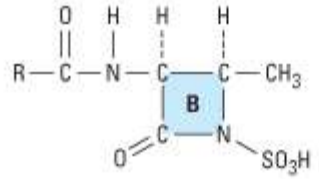
(a) **penicillins** whose official names usually include or end in “cillin”
(b) **cephalosporins** which are recognized by the inclusion of “cef” or “ceph” in their official names.
(c) **carbapenems** (e.g. meropenem, imipenem)
(d) **monobactams** (e.g. aztreonam)
(e) **beta-lactamase inhibitors** (e.g. clavulanic acid, sulbactam).



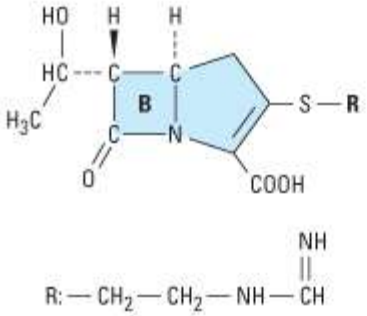
Substituted 6-aminopenicillanic acid



Substituted 7-aminocephalosporanic acid

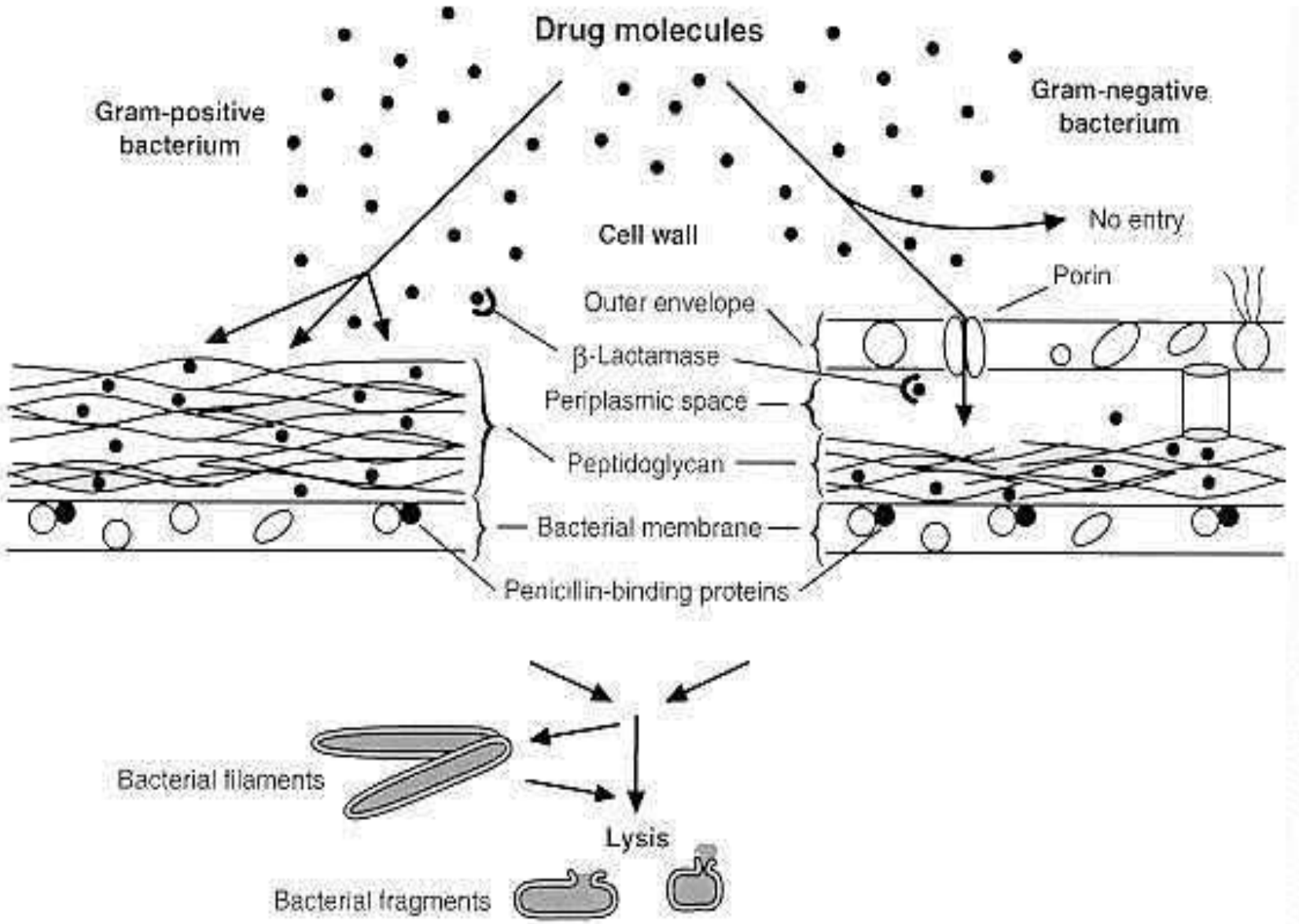


Substituted 3-amino-4-methylmonobactamic acid (aztreonam)

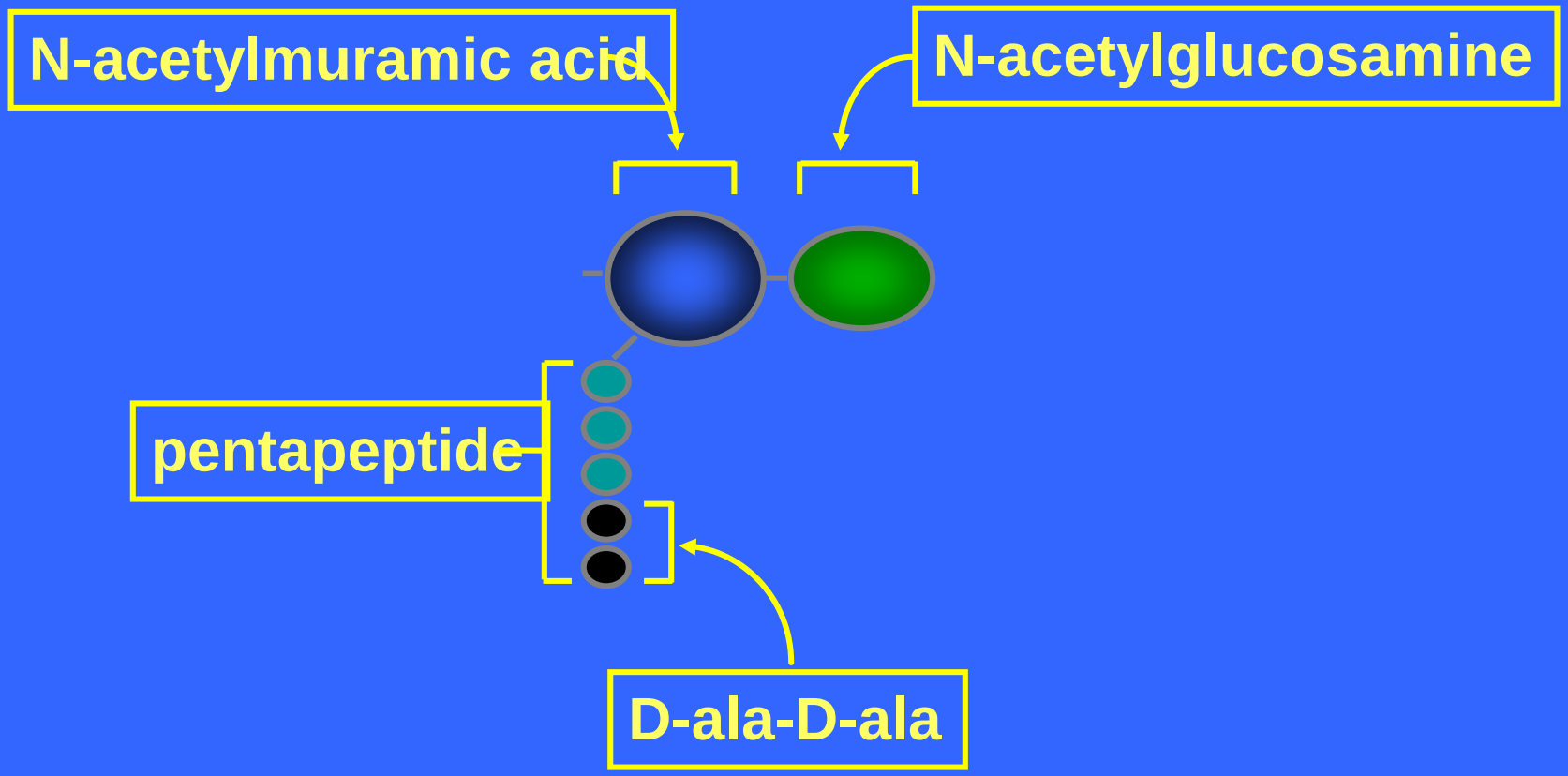


Substituted 3-hydroxyethylcarbapenemic acid (imipenem)

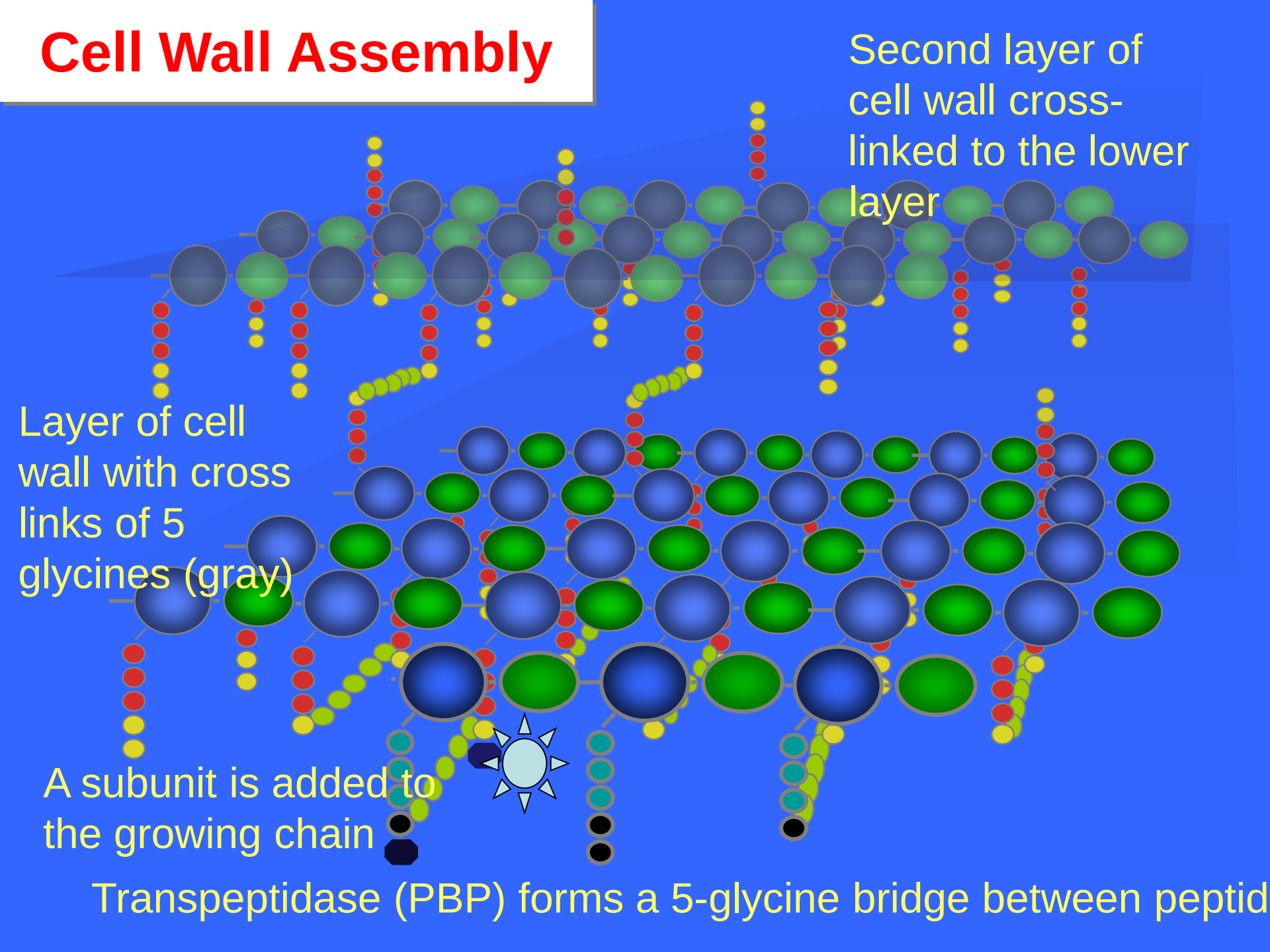
Beta-lactams effect on bacterial cell wall



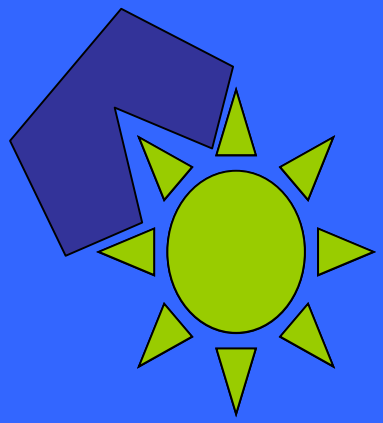
Subunits for cell wall construction



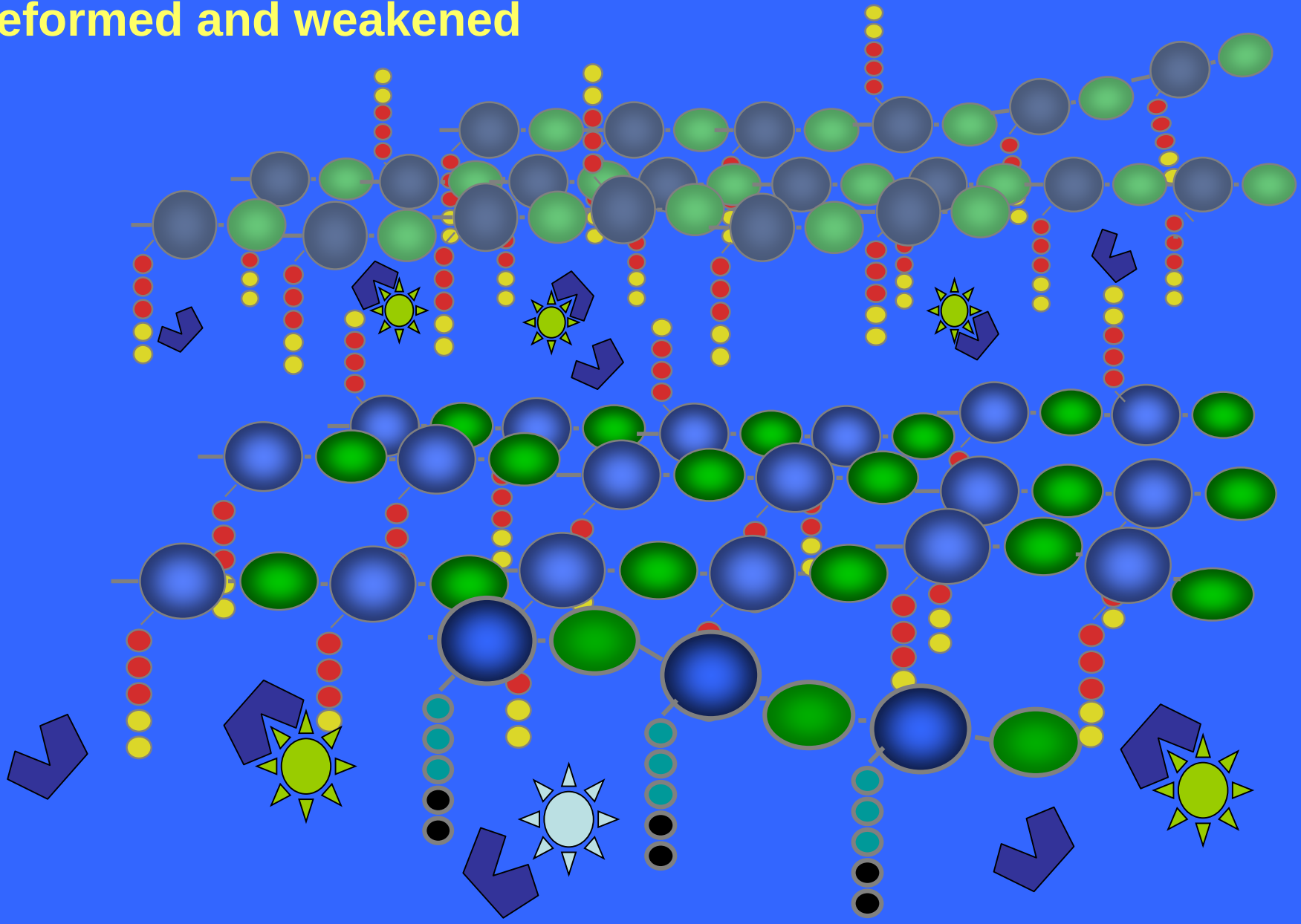
Cell Wall Assembly

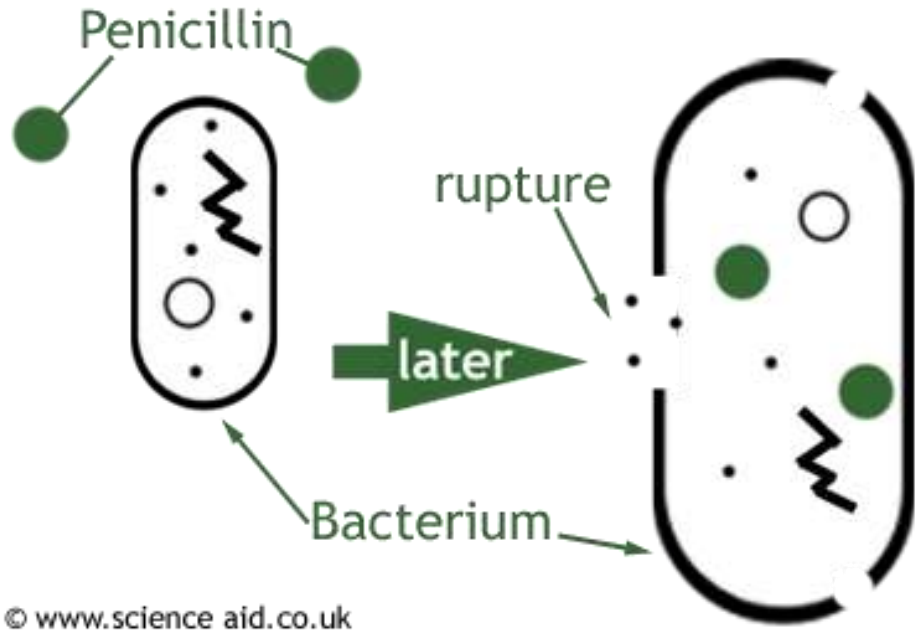


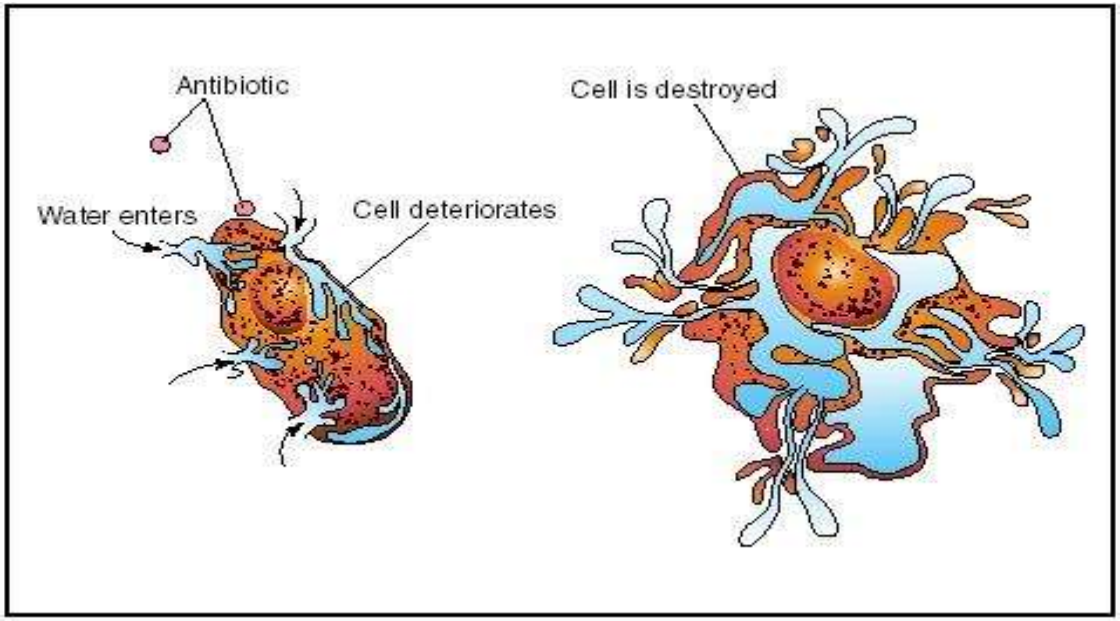
Transpeptidase, or PBP **(orange sunburst)** is bound by beta-lactam antibiotic **(light blue)** and its activity is inhibited **(turns gray)**



5-glycine crosslinking bridges cannot form in the presence of a beta-lactam, and the cell wall is deformed and weakened







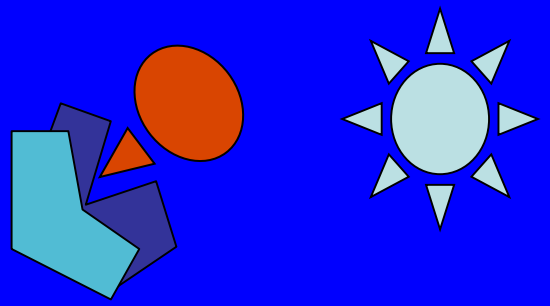
Mechanisms of beta-lactam resistance

- Drug-modifying enzymes (beta-lactamases)
  - Gram-positives(e.g., *S. aureus*) excrete the enzyme
  - Gram-negative (e.g., *E. coli*) retain the enzyme in the periplasm
- Overexpression of cell wall synthetic enzymes
  - e.g., vancomycin-intermediate *S. aureus* (VISA)
- Alteration of the PBPs so antibiotic cannot bind
  - e.g., MRSA, *S. pneumoniae*, gonococcus
- Exclusion from the site of cell wall synthesis
  - Porin mutations in the outer membrane of Gram-negative bacteria only (e.g., *Ps. aeruginosa*)

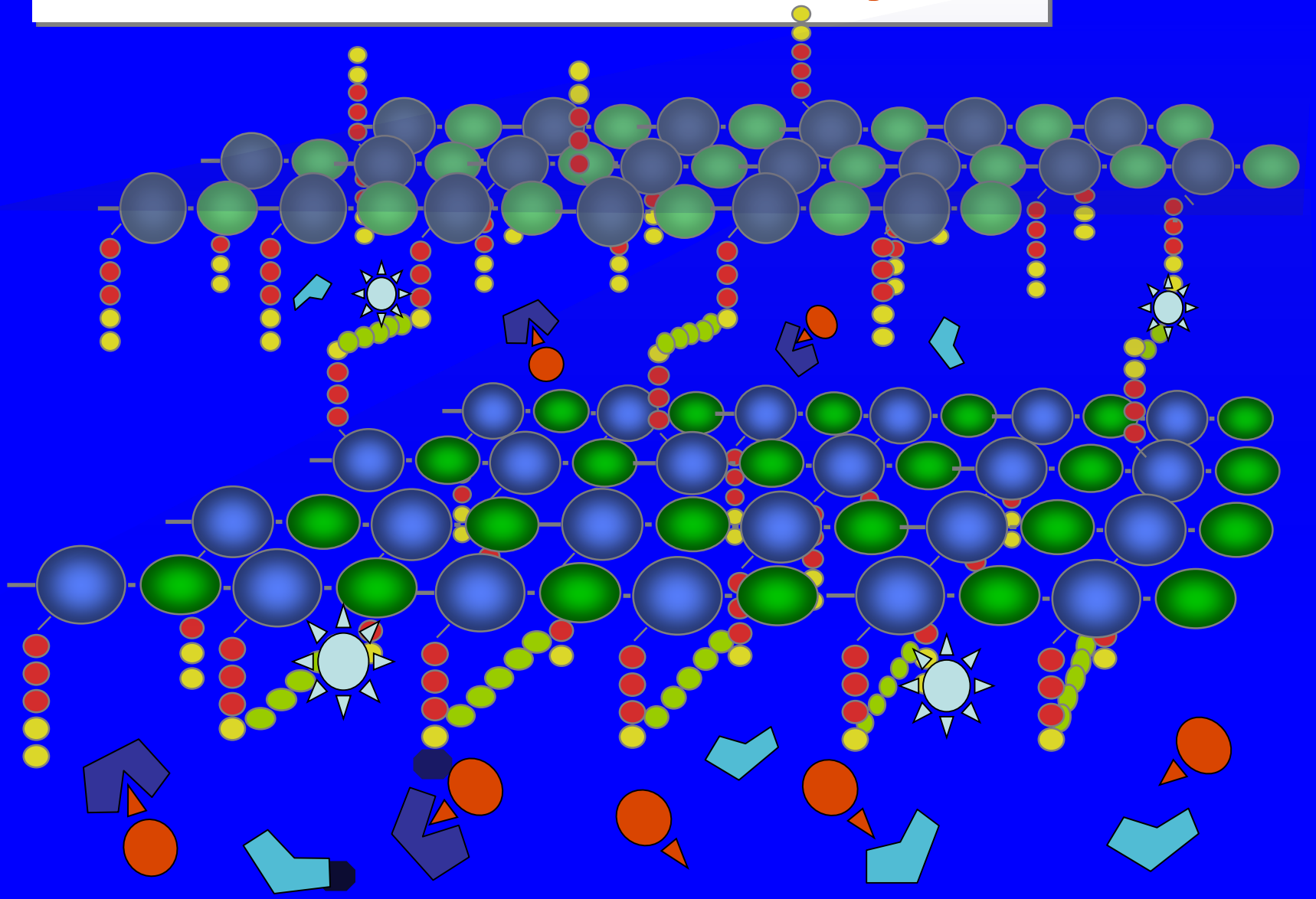
Beta-lactamases

Beta-lactamases (dark orange) bind to the antibiotics (light blue) and cleave the beta-lactam ring.

The antibiotic is no longer able to inhibit the function of PBP (orange sunburst)



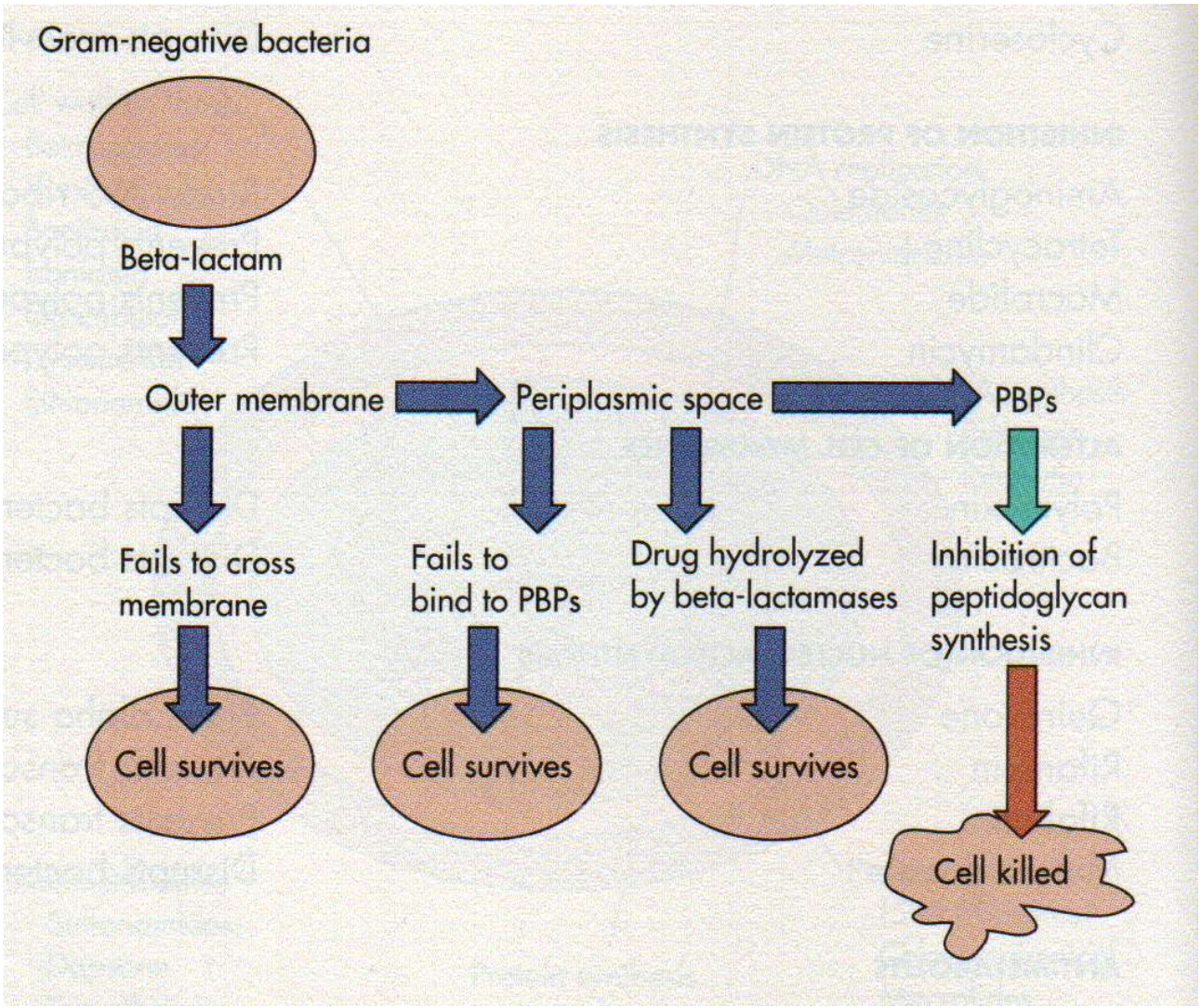
Beta-lactamase activity



Beta-lactamase inhibitors

- **Bind the beta-lactam ring irreversible**
- Clavulanic acid
  - Augmentin (amoxycillin/clavulanic acid)
- Sulbactam
  - Unasyn (ampicillin/sulbactam)
- Tazobactam
  - Tazocin (piperacillin/tazobactam)

Resistance to β-Lactams – Gram negative

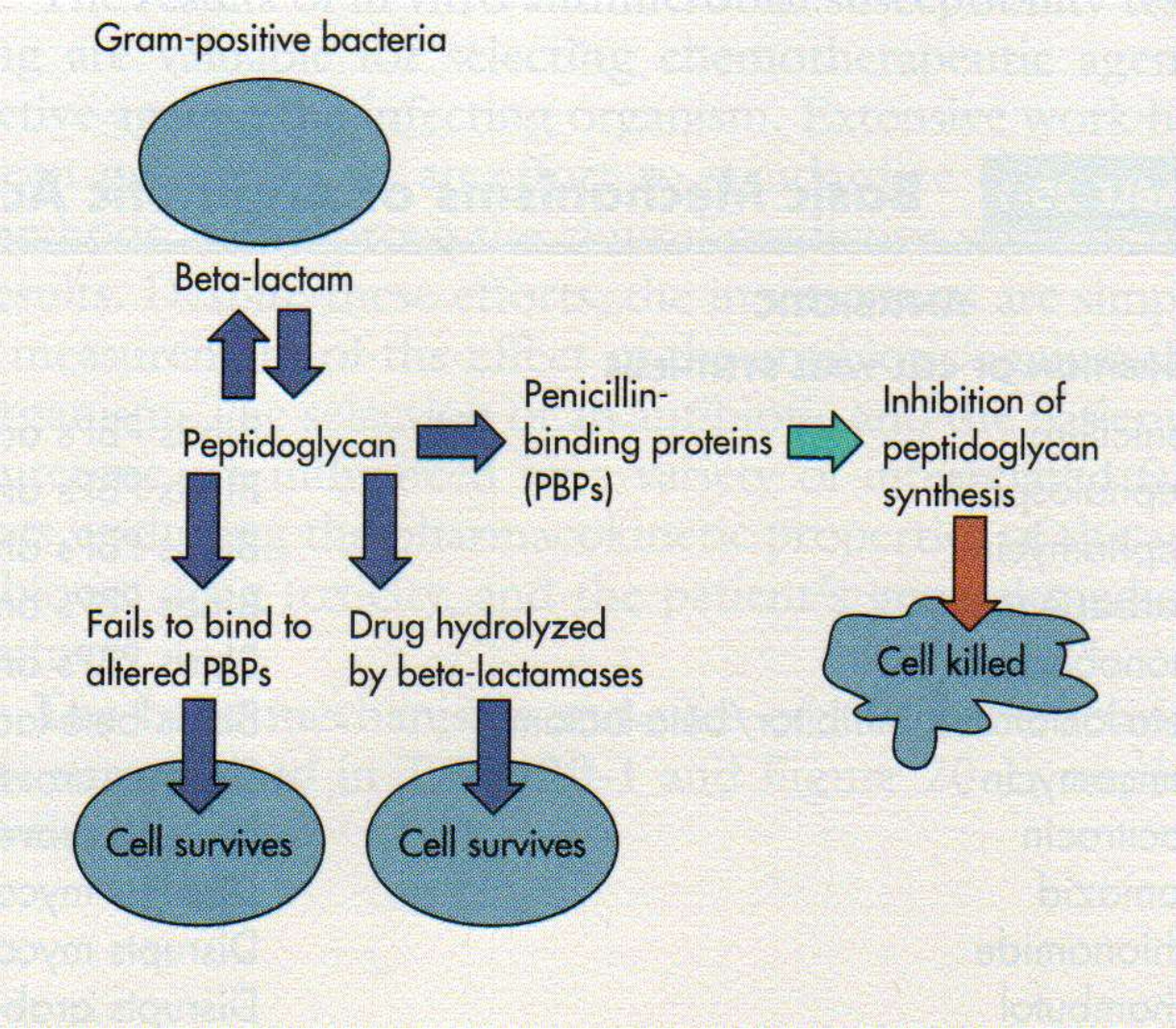


Beta-lactamases

- Extended-spectrum beta-lactamase ESBL
  - Hydrolyze: penicillins, cephalosporins
  - NOT hydrolyze: carbapenems, monobactams
  - Beta-lactamase inhibitors: clavulanic acid, sulbactam, tazobactam will inhibit!
  - Gram-negatíve bacteria

- Metallo beta-lactamase (MBL)
  - Hydrolyze: CARBAPENEMS
  - Gram-negatíve bacteria

Resistance to β-Lactams – Gram positive



Methicillin resistant Staphylococcus aureus

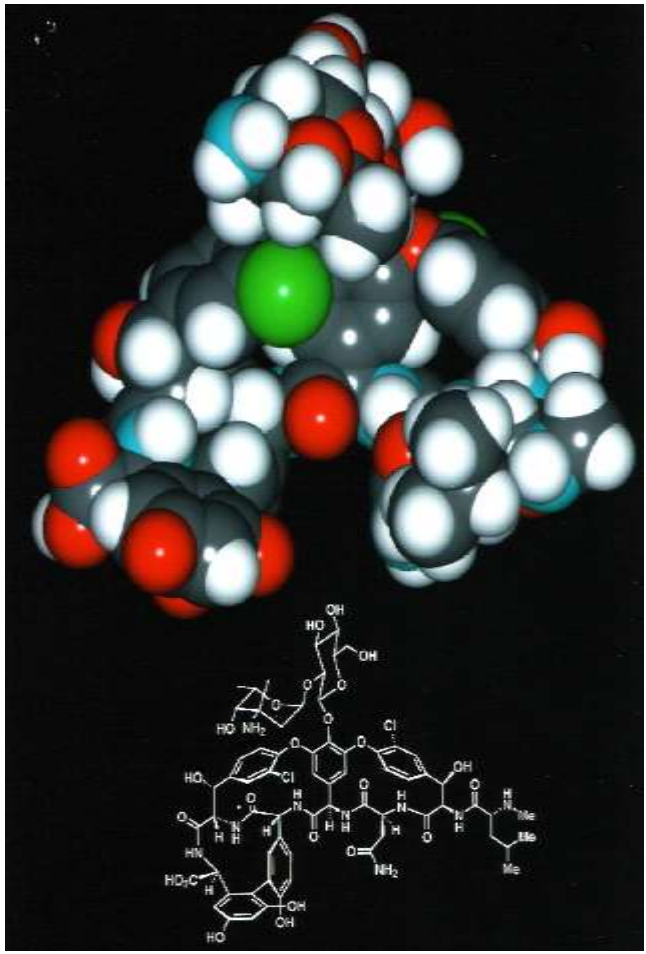
MRSA

- Penicillin-binding proteins (PBPk) - structure modification
- Resistance to **ALL BETA-LACTAM ANTIBIOTICS**:
  - Penicillins
  - Cephalosporins
  - Carbapenems
  - Even to Beta-lactamase inhibitors

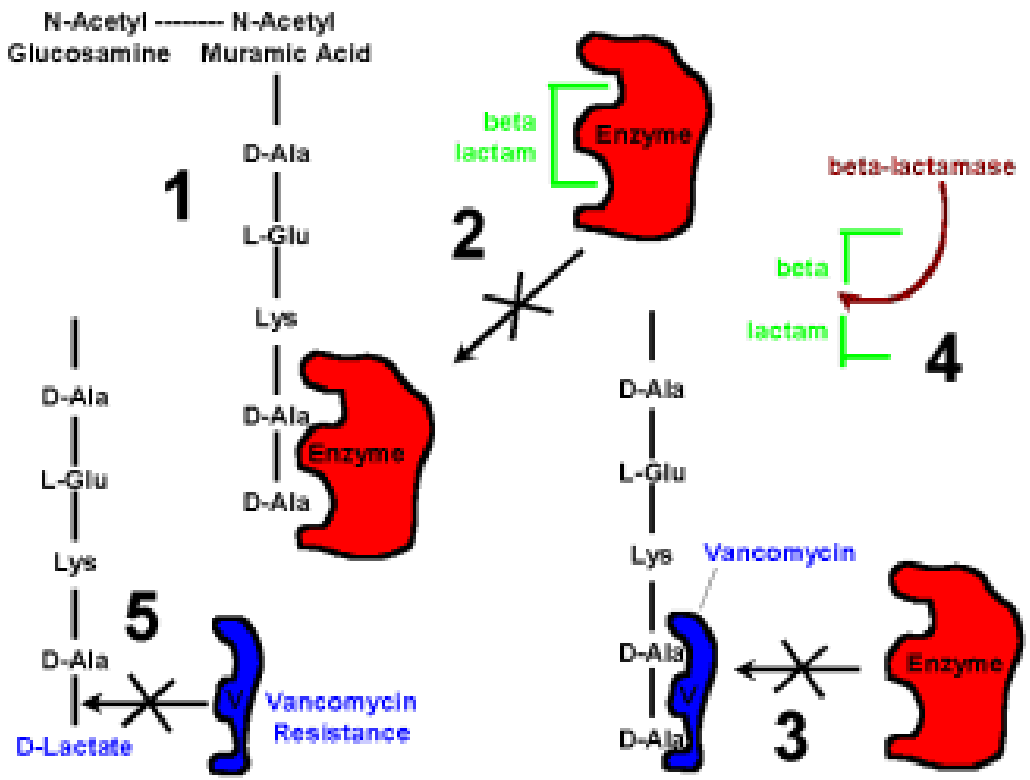
Glycopeptides: Vancomycin, Teicoplanin

Komplex Effect - Glikopeptides

- Vancomycin, Teicoplanin
  - interfere with Peptidoglicansynthese
  - destroy the cytoplasmic membrane
  - prevent RNA synthesis
- can not go through Gram-negative cell wall
- Only for Gram-positive bacteria

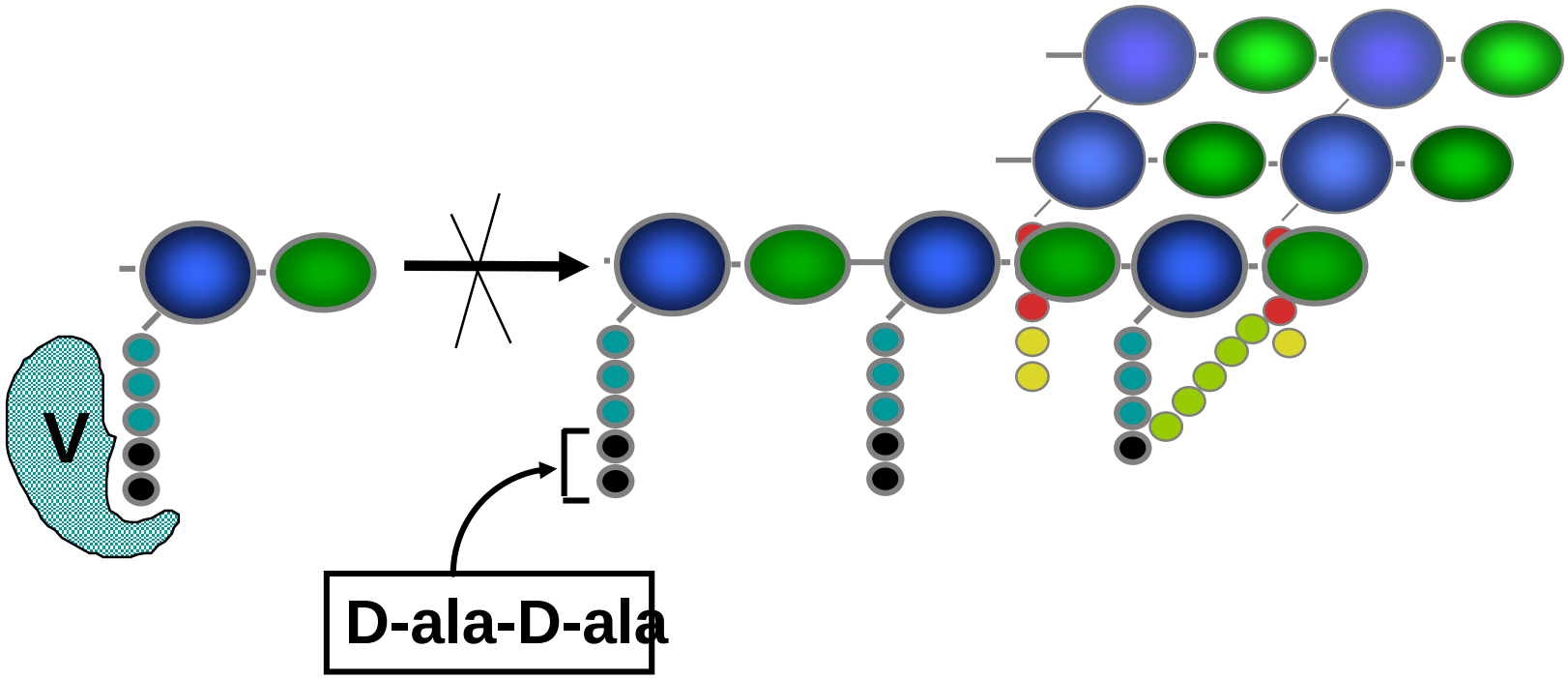


Inhibition of peptidoglycan cross-linking by Beta-Lactams and Vancomycin and mechanisms of resistance.



1. Transpeptidase enzyme binds to D-Ala-D-Ala for cross-linking.
2. Beta-lactam antibiotic binds to transpeptidase inhibiting cross-linking.
3. Vancomycin binds to D-Ala-D-Ala preventing binding of enzyme.
4. Beta-lactamase cleaves beta-lactam antibiotic.
5. Changing terminal D-Ala to D-Lactate prevents vancomycin binding.

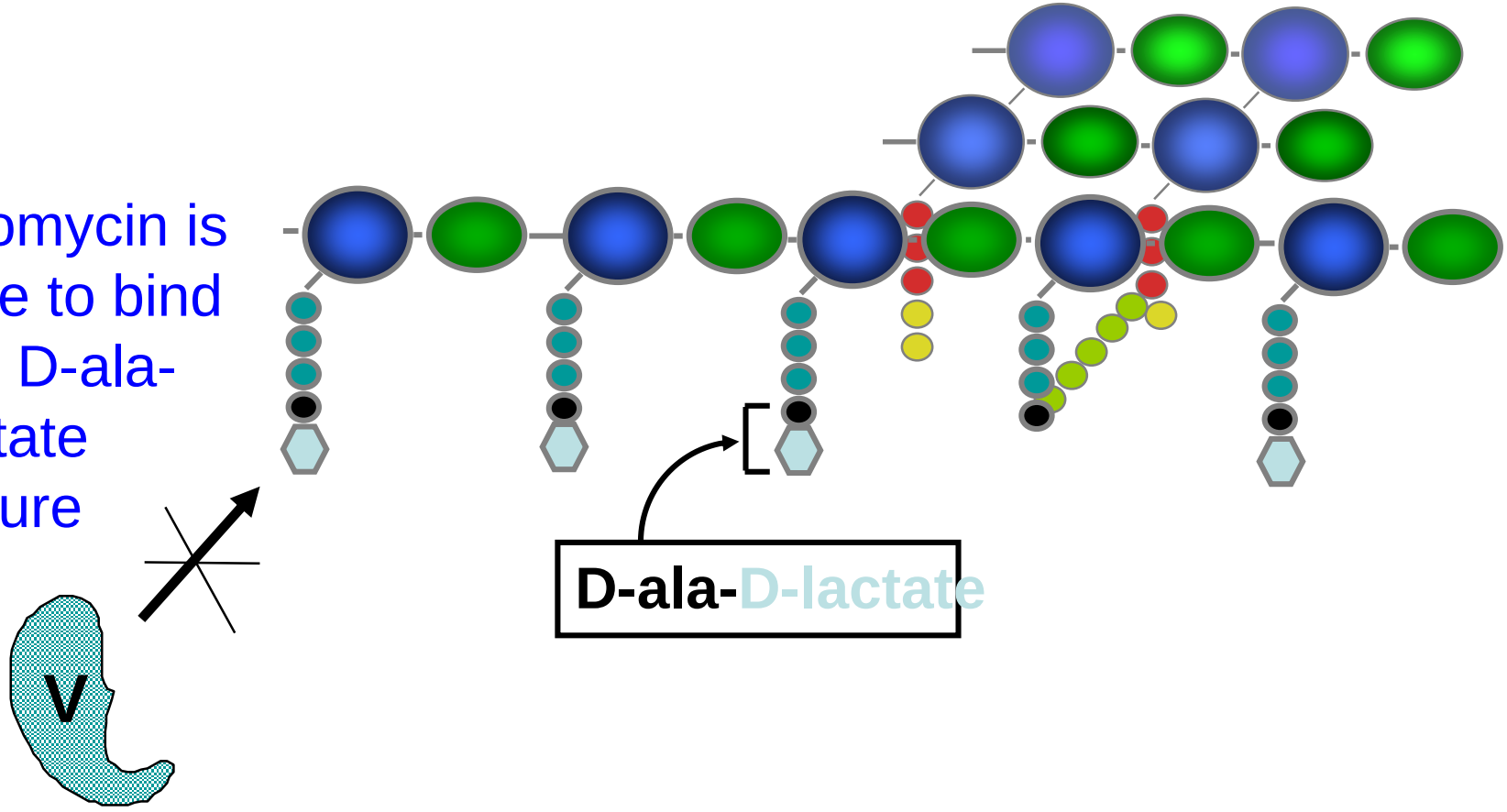
Mechanism of vancomycin action



Mechanism of vancomycin resistance

Vancomycin resistant Enterococcus (VRE)

Vancomycin is unable to bind to the D-ala-D-lactate structure



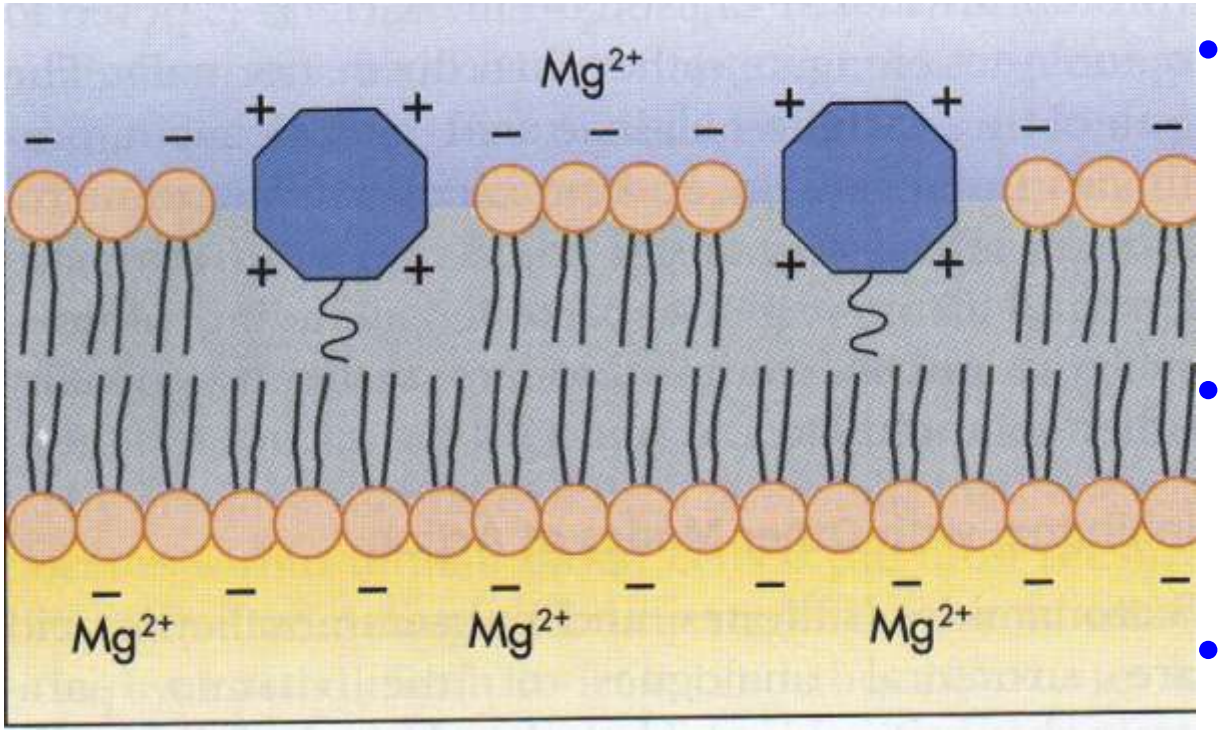
ANTIBIOTICS AFFECTING CELL MEMBRANES

- **Polypeptides**
  - **Surface active amphipathic agents.**
  - **Interact strongly with phospholipids and disrupt the structure of cell membranes.**
- **Daptomycin**
  - **Depolarizes the cell membrane**

Polypeptids

- Disintegration of the outer membrane
- Narrow spectrum only against G negative (except Proteus, Neisseria)
- Bactericidal Antibiotics
- kidney toxicity
- Intestinal decontamination-per os are not absorbed
- Eye, ear drops, wound infections
- for example:. polymyxin B, colistin (im, iv..)

Mechanism of Action
ALTERATION OF CELL MEMBRANES



- binds to lipopolysaccharide on outer cell wall of GNR;
- permeability change in cell envelope;
- leakage of cell content.

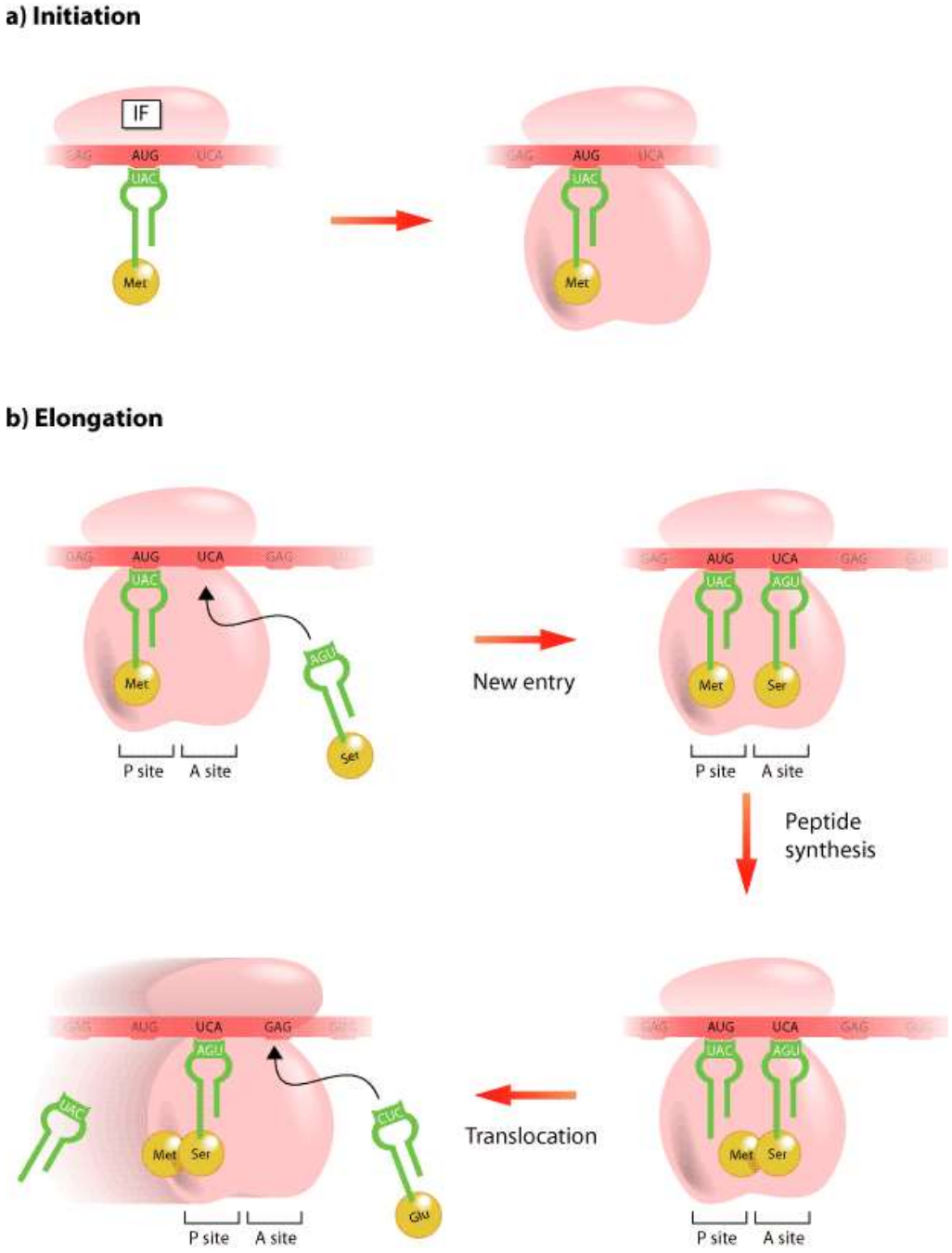
Colistin

- Spectrum: aerobic gram-negative rods, including *Acinetobacter*, *Ps. aeruginosa*, *Stenotrophomonas*.
- NOT active against: *Burkholderia*, *Proteus*, *Serratia*, *Brucella*, gram-negative anaerobes, gram-positive cocci
- Adverse effects: Neurotoxicity – dizziness, weakness, vertigo, visual changes, confusion, ataxia.

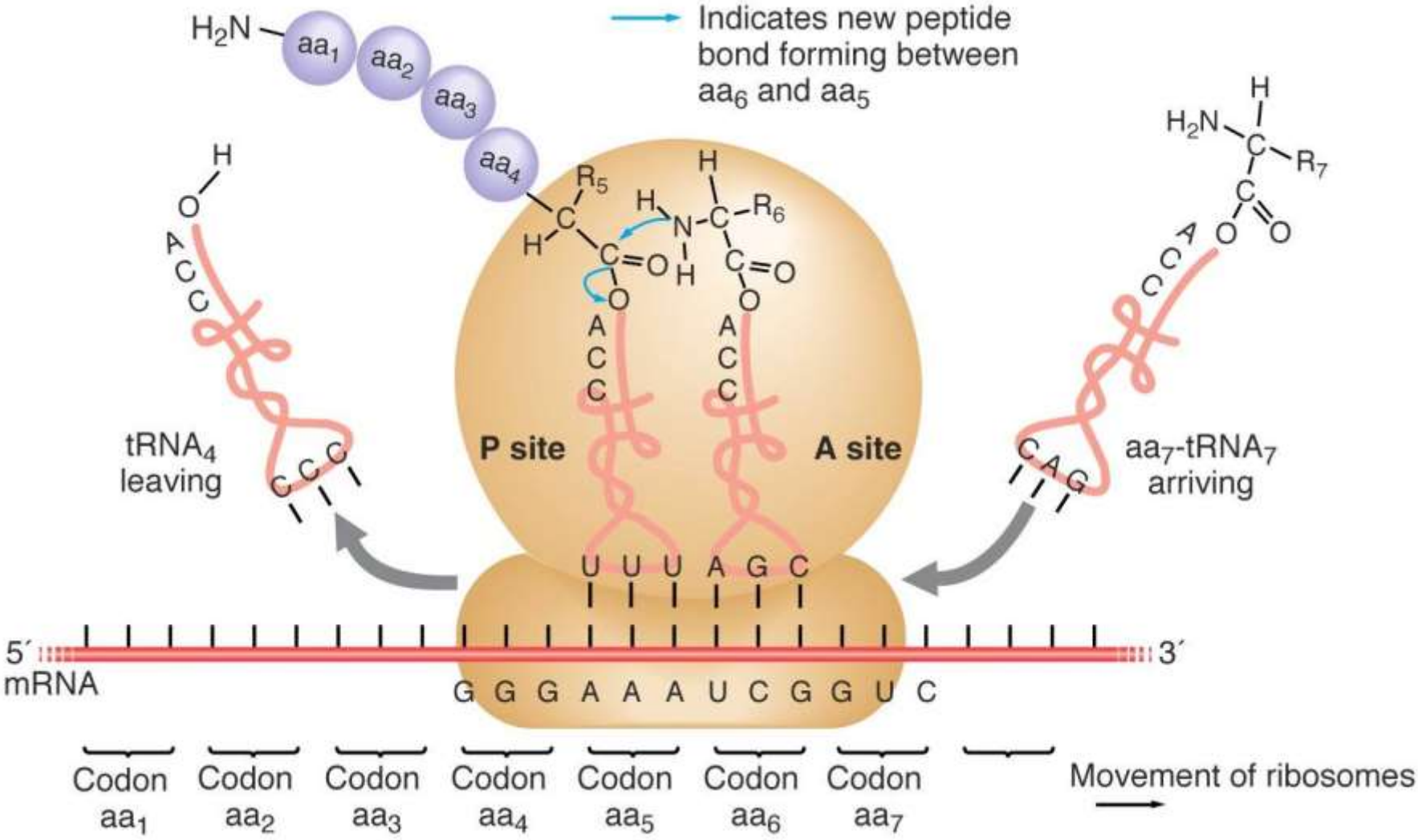
ANTIBIOTICS INHIBITING PROTEIN SYNTHESIS

- Macrolides
- Clindamycin
- Linezolid
- Streptogramins
- Chloramphenicol
- Tetracyclines
- Aminoglycosides

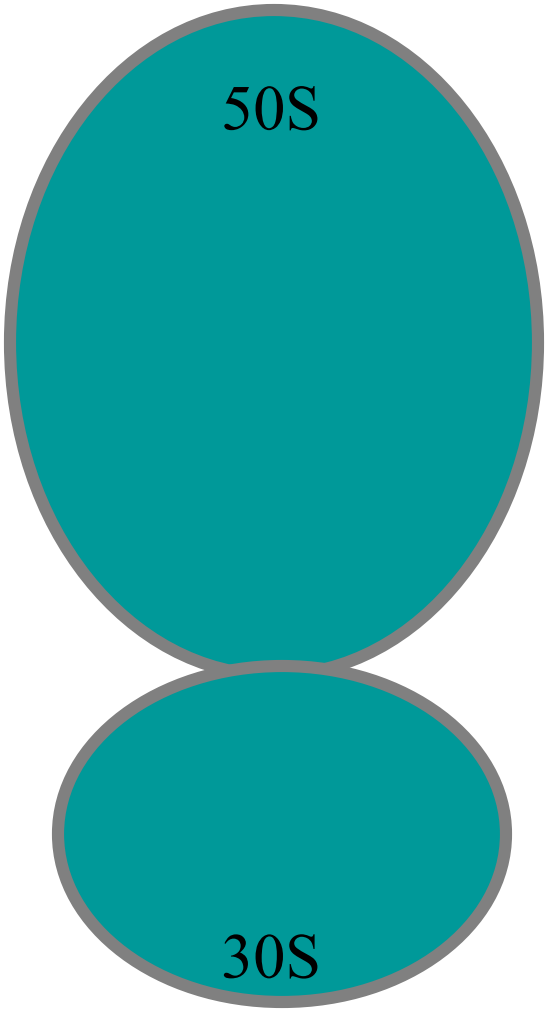
Proteinsyntesis



www.scq.ubc.ca/.../2006/08/protein-synthesis.gif

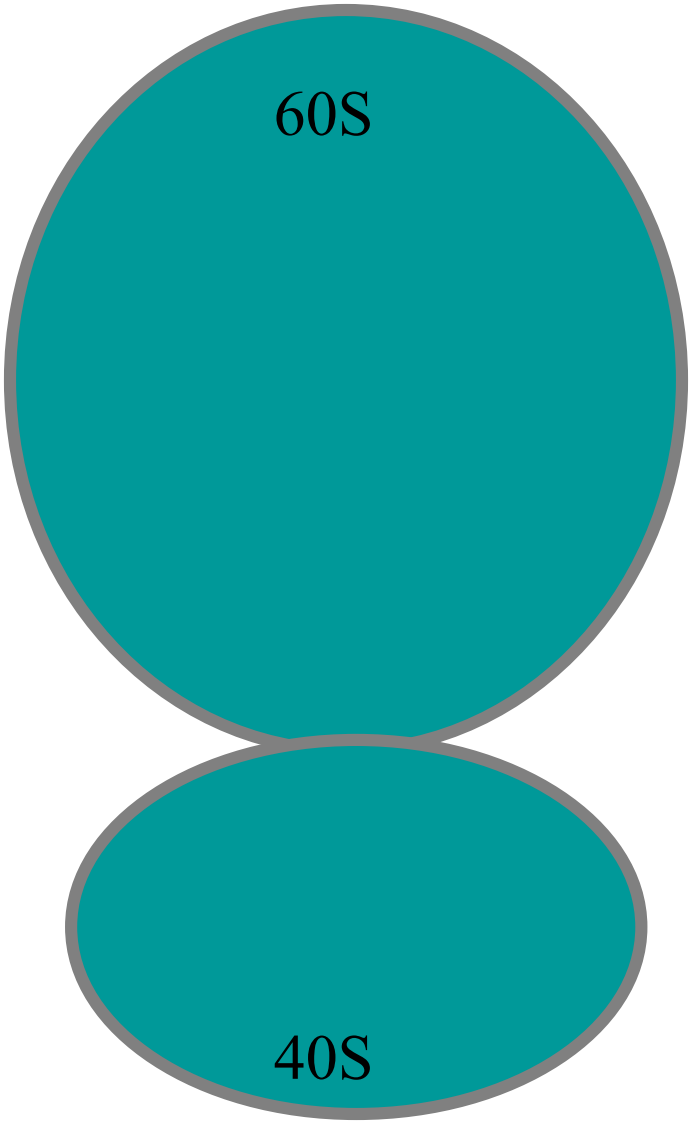

Indicates new peptide bond forming between aa6 and aa5
H2N
aa1
aa2
aa3
aa4
tRNA4 leaving
P site
A site
aa7-tRNA7 arriving
5′
mRNA
3′
U U U A G C
G G G A A A U C G G U C
Codon aa1
Codon aa2
Codon aa3
Codon aa4
Codon aa5
Codon aa6
Codon aa7
Movement of ribosomes

Procaryotic Ribosome



70S--
M.W.2,500,000

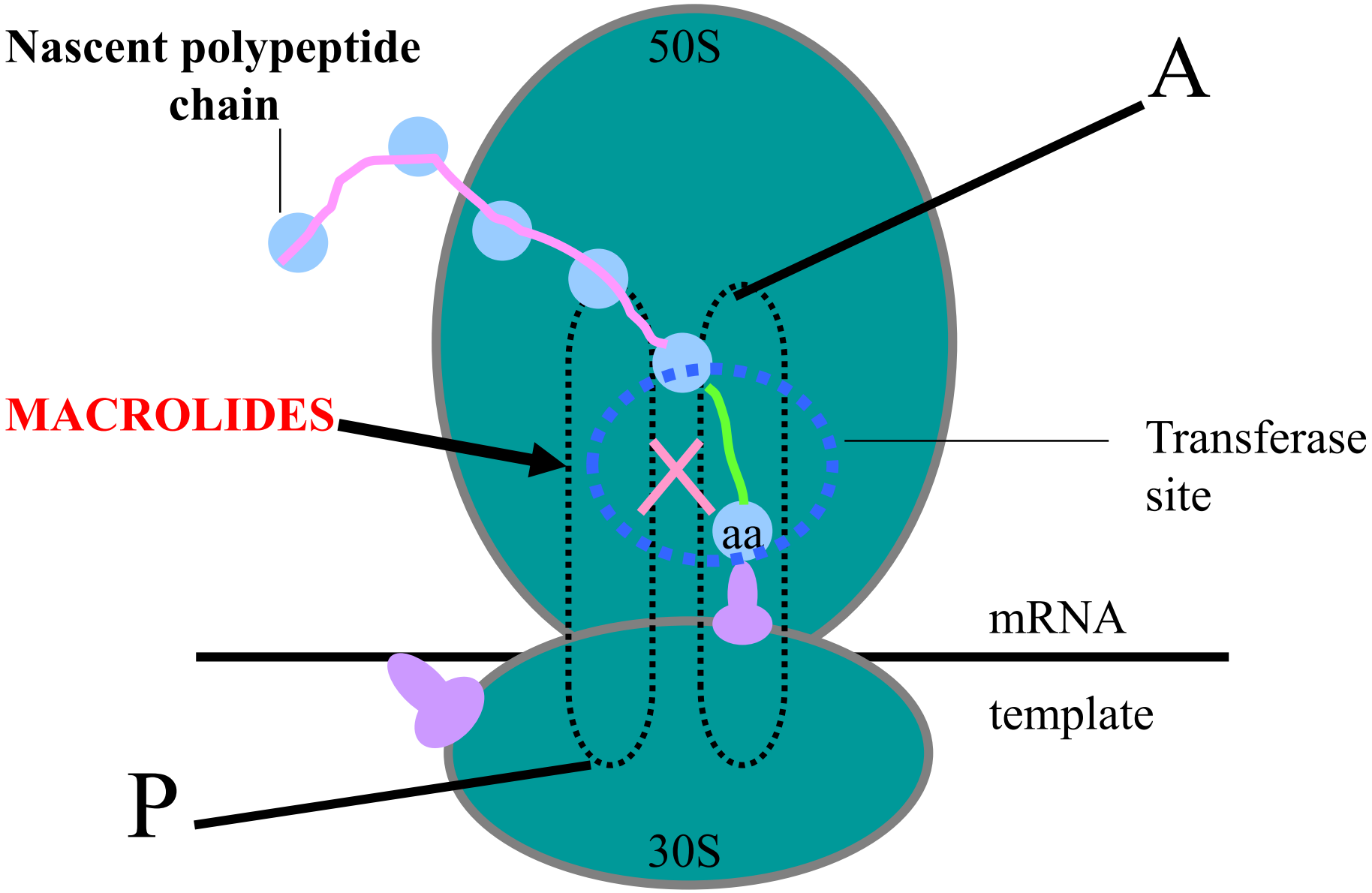
Eucaryotic Ribosome



80S--M.W.
4,200,000

Antibiotics binding to the 50S ribosomal subunit and inhibiting protein synthesis

- Erythromycin and other macrolides
- Chloramphenicol
- Linezolid
- Streptogramins



TRANSLOCATION

Macrolides:

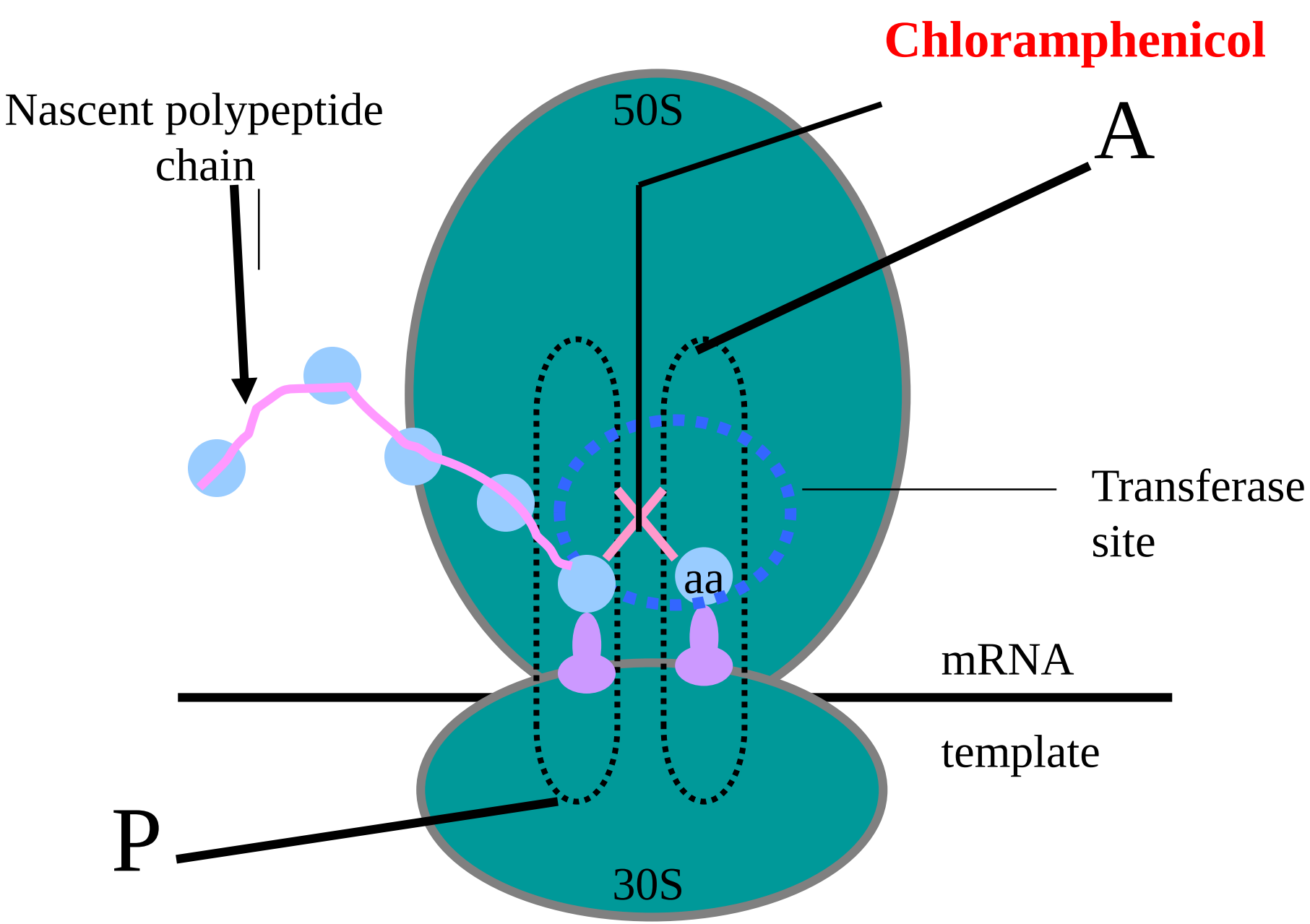
Erythromycin, Clarithromycin, Azithromycin

- Use:
  - Broad spectrum against gram positives including Staph aureus (MSSA)
  - Good for atypical oganism such as Mycoplasma, Chlamydia, Legionella
  - Covers *N. gonorrhea, H. influenzae, Legionella*
- Caution:
  - can interact with statin to cause myopathy
  - Can cause Qt prolongation
- Side effects:
  - GI upset

Makrolides and lyncosamides

- Prevent the movement of mRNA to the 50S ribosoma unit
- Bacteriostatic
- little toxicity
- Effective against intracellular living bacteria, anaerobic streptococci, against Campylobacter
  .: eg erythromycin, azithromycin, Clyndamycin

- Resistance to macrolides
  - Encoded on the Kromosomen: Change in ribosoma unit
  - Encoded on plasmids: efflux

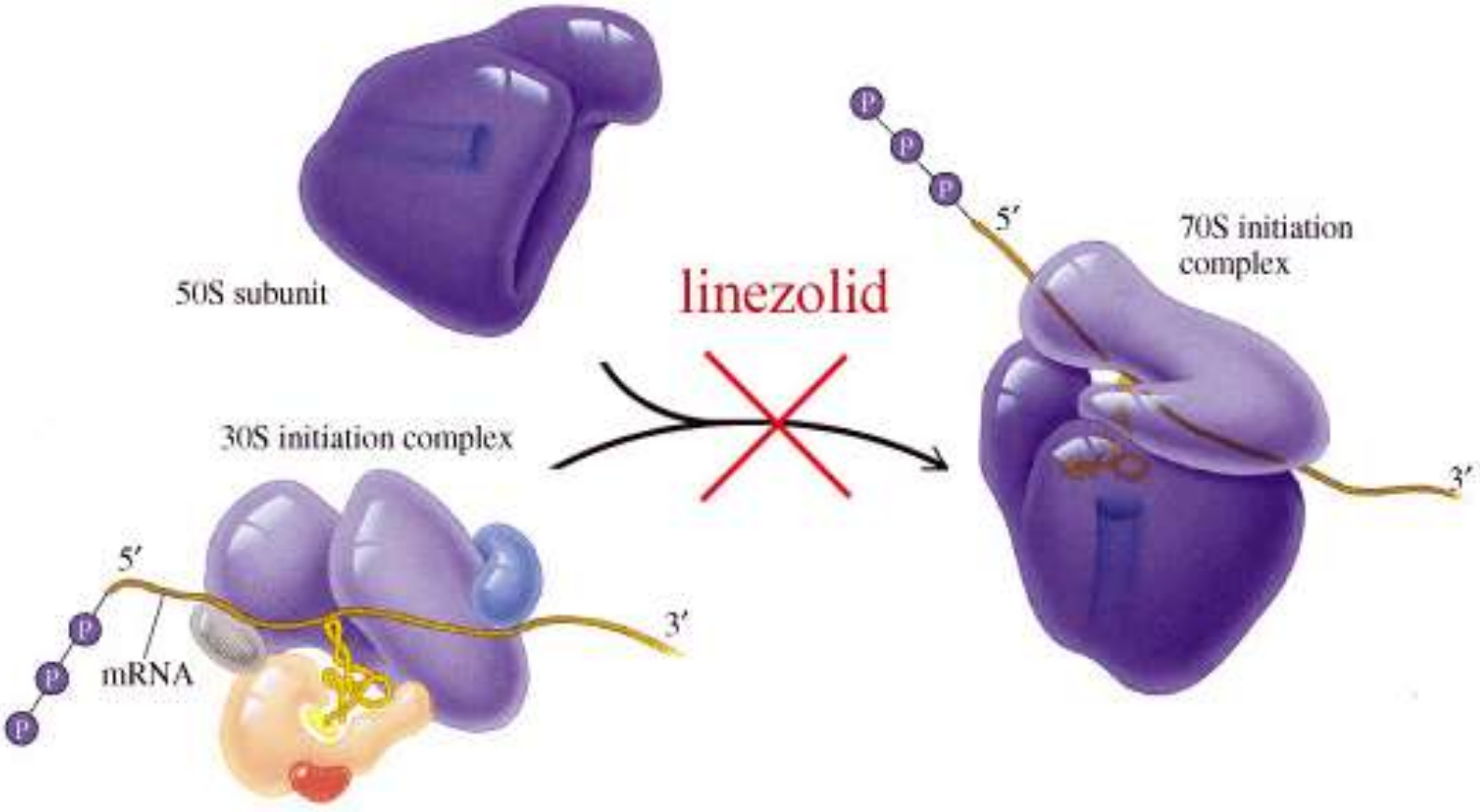


Mechanism of action of Chloramphenicol

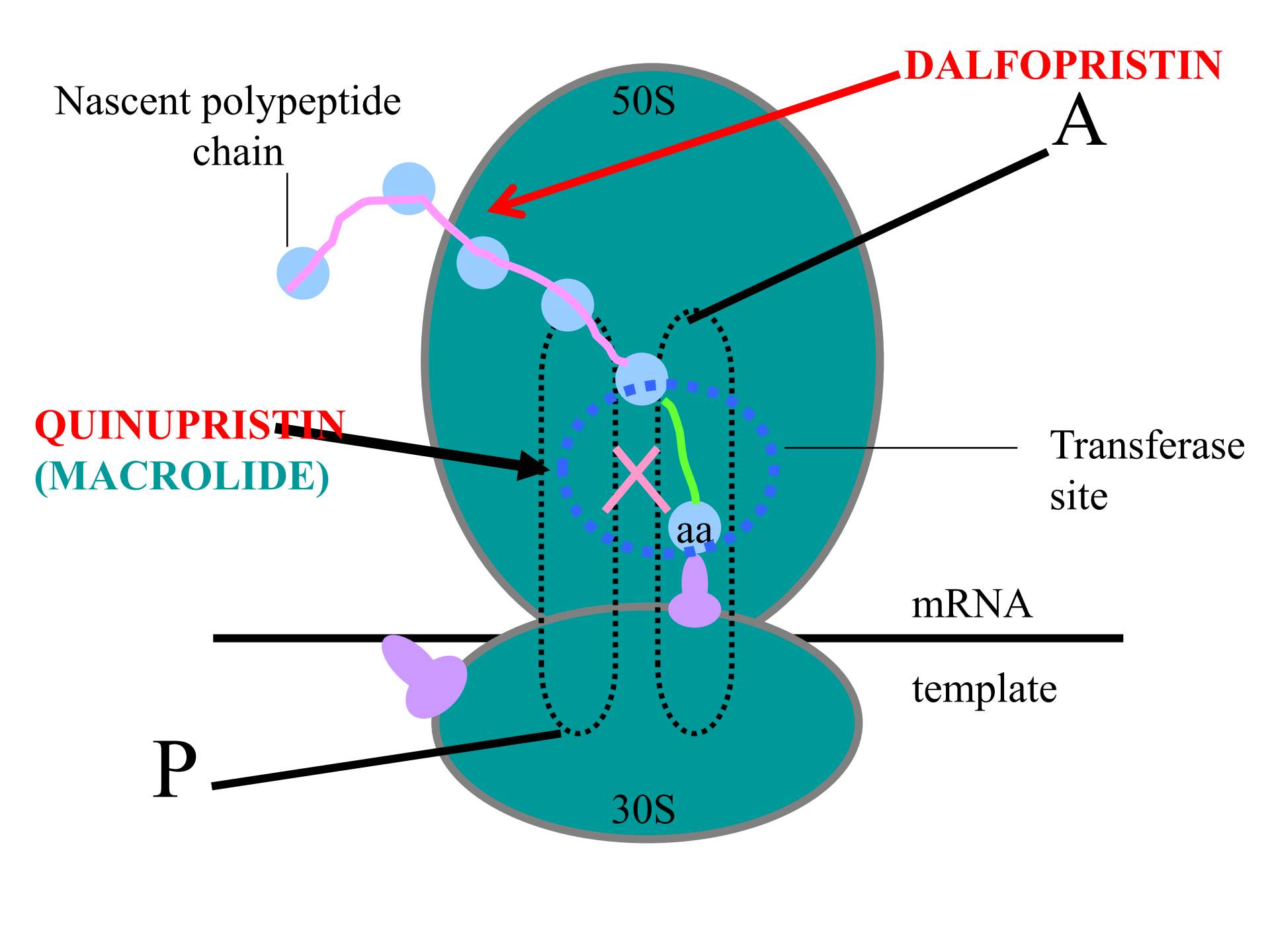
Chloramphenicol

- Interfere with the attachment of tRNA on the 50S ribosome unit
- Bacteriostatic
- broad-spectrum
- Systemic we only use against *H. influenzae* meningitis, and in intraocular infections
- Very toxic
  - Destroys the blood cell is being made (Panzytopenia)
  - Gray Syndroma in neonates with liver damage
  - Dysbacteriosis, necrotising ulcerative colitis

Linezolid



- Inhibits the formation of 70S Initiationkomplex
- August 2005 „Molekule of the Month”
- For Gram-positive cocci

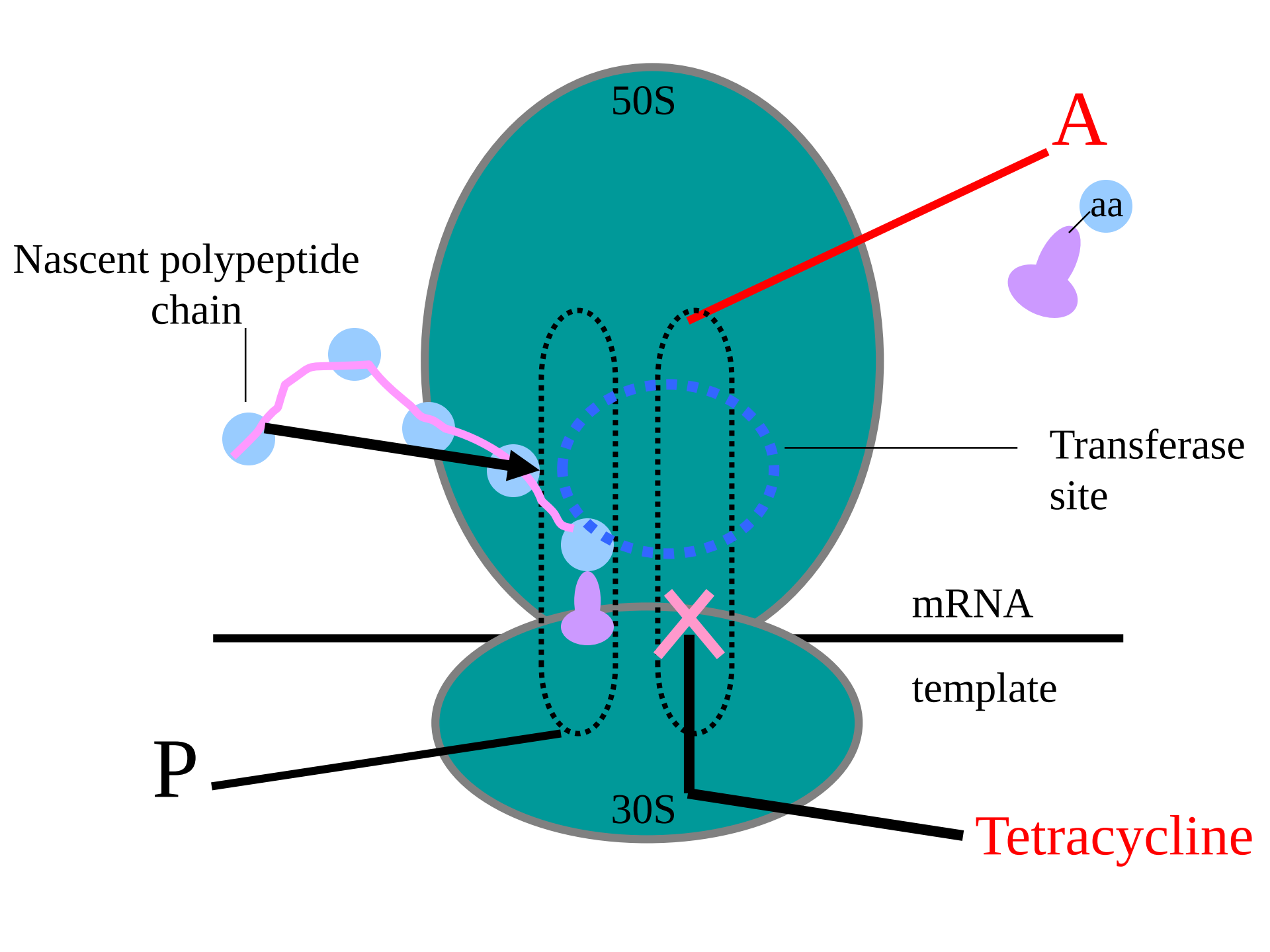

DALFOPRISTIN
Nascent polypeptide chain
50S
A
QUINUPRISTIN
(MACROLIDE)
Transferase site
aa
mRNA
template
P
30S

Quinupristin-Dalfopristin

- Semisynthetic Streptogramine Derivate
- Inhibition of Peptidyltransferase on the 50 S
- Q. cause conformation changes
- D. better binding
- Stronger effect= Baktericid
- Gram-positive cocci MRSA

Antibiotics binding to the 30S ribosomal subunit and inhibiting protein synthesis

- **Aminoglycosides**
- **Tetracyclines**


50S
A
aa
Nascent polypeptide chain
Transferase site
mRNA
template
P
30S
Tetracycline

Aminoglycosides

- Inhibit the transcription of the 30S ribosome unit
- The active transport of antibiotics need O2 The anaerobic bacteria are genetically resistant to aminoglycosides
- The first antibiotic was only the streptomycin against Mycobacterium
  - Netilmicin, tobramycin, amikacin gentamicin we can locally and systematically use also
  - Neomycin we only use in eye drops

Aminoglycoside resistance

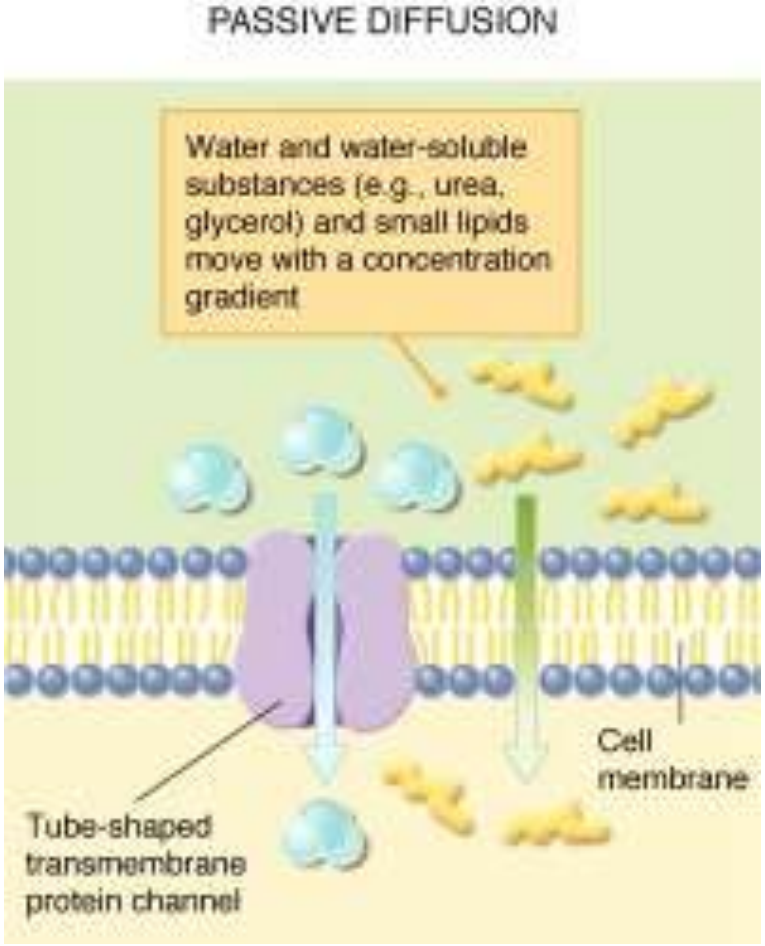
- Encoded on the chromosome
- The structure of 30S ribosome unit is changed
  - high level resistance in enterococci
- Permeations inhibition by anaerobic metabolism
  - Low level resistance in enterococci
- Combination with cell wall synthesis inhibitors in endocarditis
  - Encoded on plasmids
  - Antibiotics destroy the enzymes inactivation / structural modification by
    - Acetylation
    - Adenylation
    - phosphorylation

Tetracycline

- Prevents the attachment of tRNA on the 30S ribosome unit
- Bacteriostatic
- Broad-spectrum activity
  - Includes aerobic G+ and G-, atypicals [Rickettsia spp, treponema spp, chlamydia spp, and others]
  - Little to no effect on fungi or viruses

- Tigecycline
  - 70% of Hungarian bacteria are resistant
    - efflux pumps
    - Stabilization of ribosome-tRNA
- Tetracycline, Doxycycline, Minocycline
- New derivatives: tigecycline

Mechanism of Action



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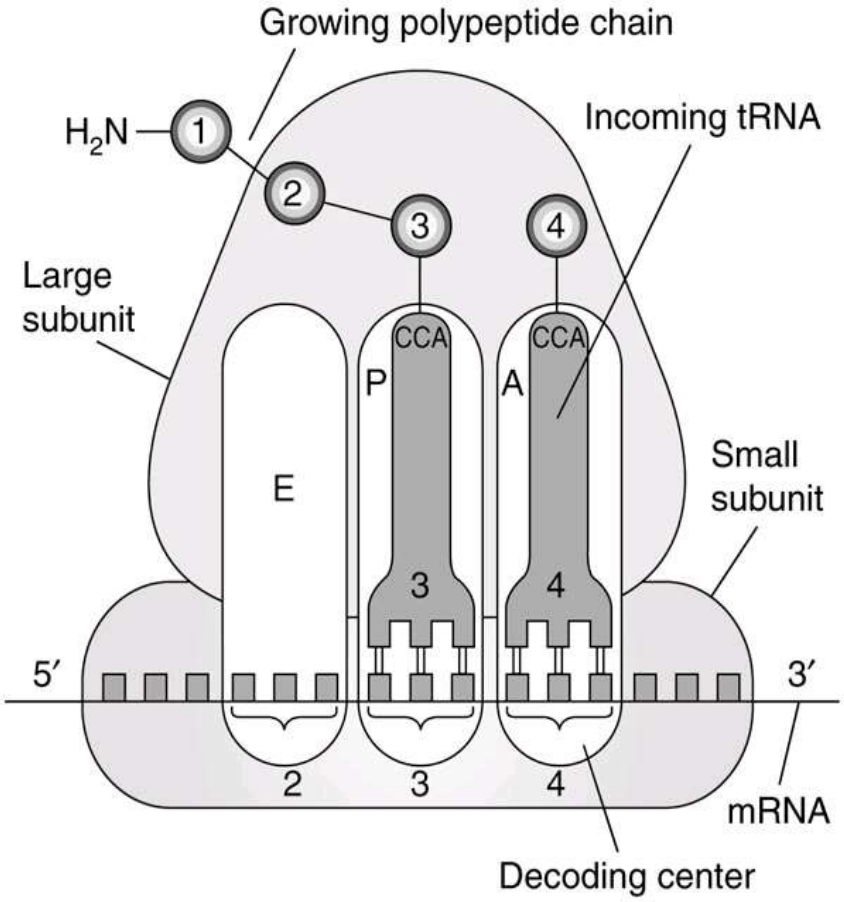
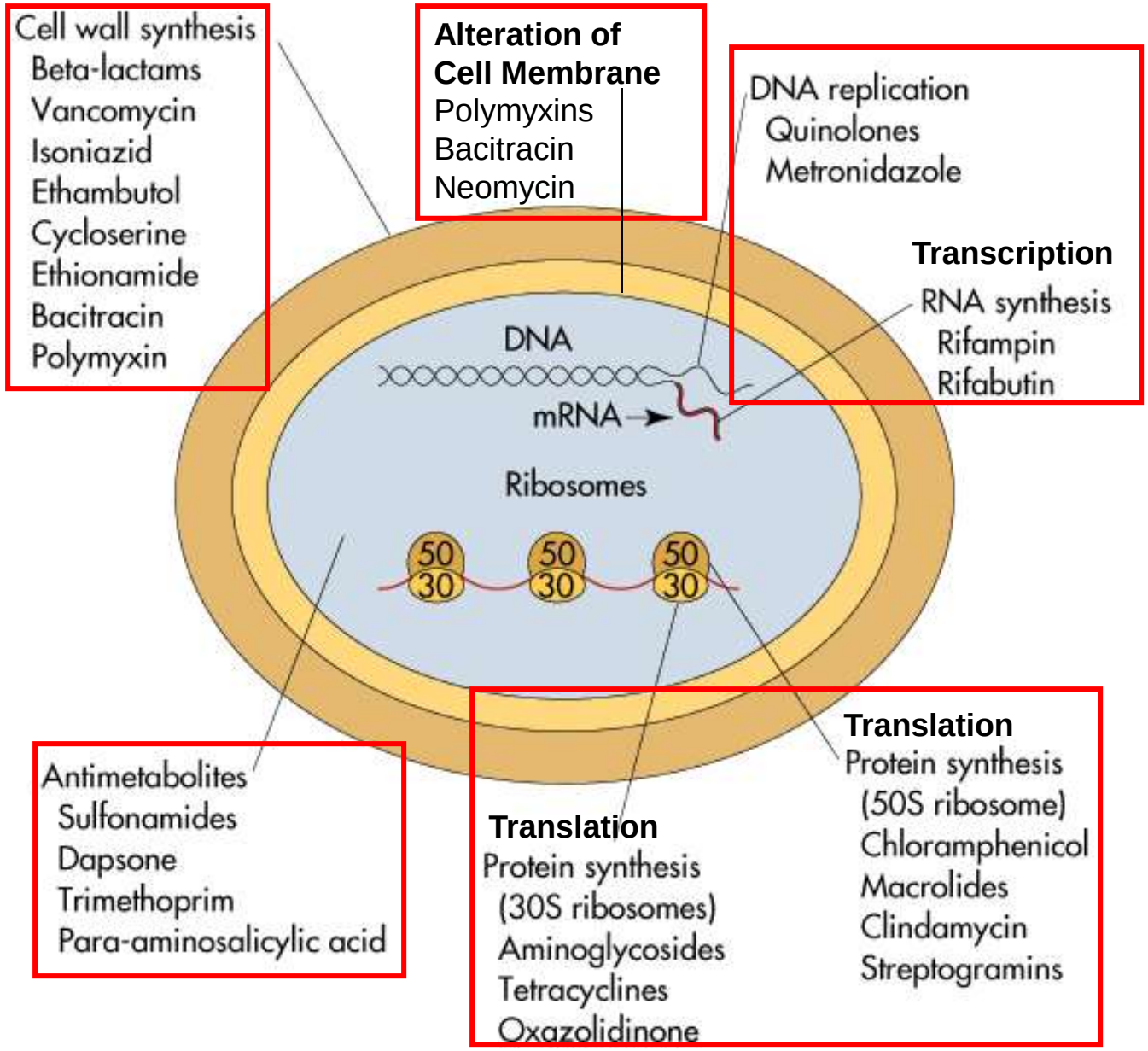


- Once inside the cell…
  - Bind 30S ribosomal subunit
  - Blocks binding of aminoacyl-tRNA to acceptor site on mRNA-ribosome complex
  - Protein synthesis is inhibited = bacteriostatic effect

Mupirocin

- Prevents the attachment of Izoleucin –tRNS
- produced by Pseudomonas fluorescens
- Active against only staphylococci and streptococci
- Lokale effect against MRSA (Baktroban)
- Topical treatment of impetigo

Antibiotic Mechanisms of Action

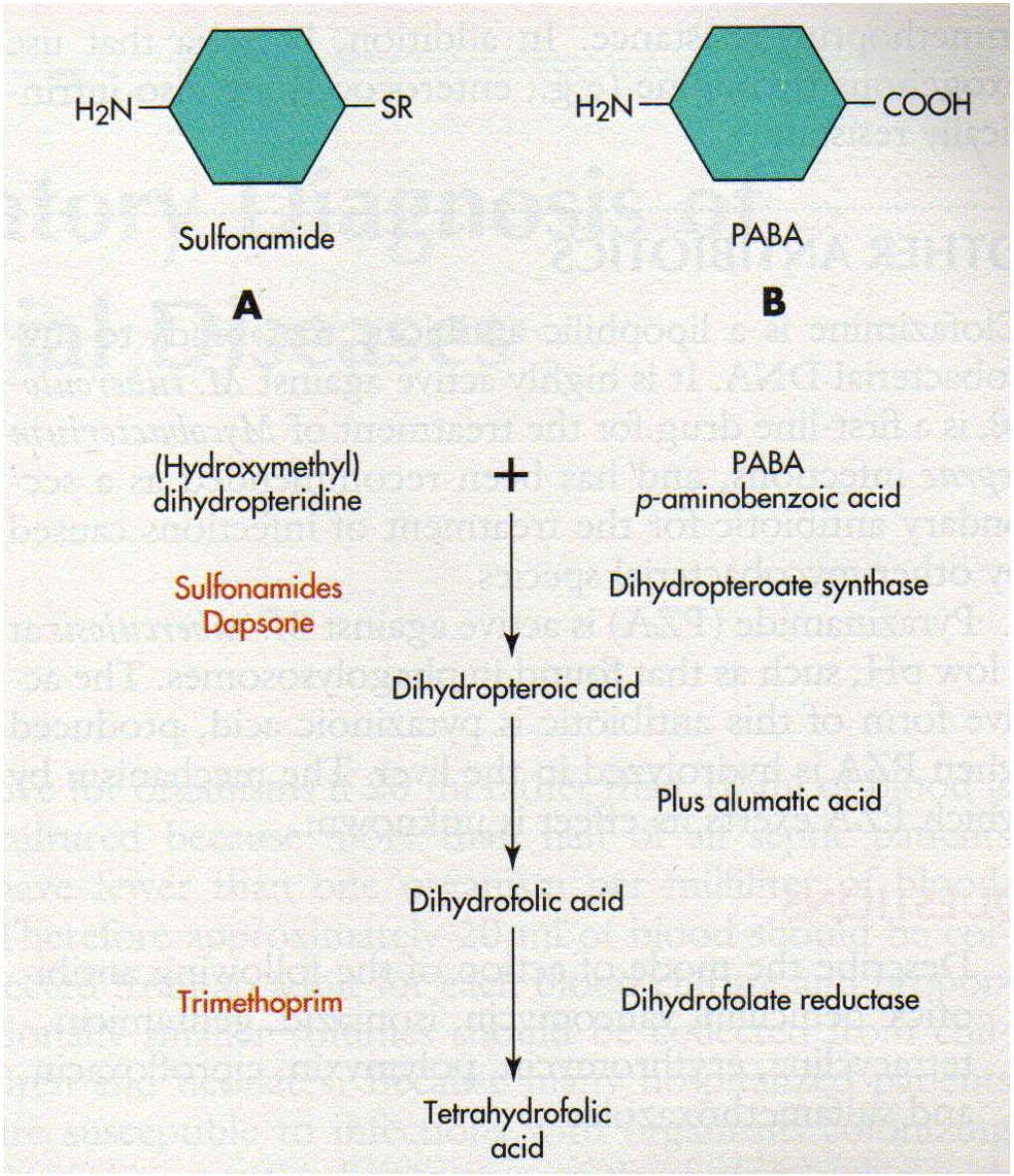


ANTIBIOTICS ACTING AS ANTIMETABOLITES

- **Sulfonamides**
- **Trimethoprim plus sulfamethoxazole**

Mechanism of Action

ANTIMETABOLITE ACTION



Trimethoprime/Sulphamethoxazole

- Good activity against Gr (+) and Gr (-) organisms: MRSA, very active against PCP. Covers *Stenotrophomonas maltophila, Nocardia,* and enteric gram-negative rods.
- Exceptions: *Pseudomonas aeruginosa, Group A strep, enterococcus*, Gr (-) anaerobes.
- Toxicity: GI upset, rash can progress to SJS and TEN, thrombocytopenia, leucopenia, hepatitis; hyperkalemia
- SMX:TMP is a 5:1 ratio, in oral and IV dosage forms.

SULFONAMIDE-RESISTANCE

- **Results from multiple mechanisms.**
- **Altered dihydropteroate synthetase.**
- **Cross-resistance among all sulfonamides.**

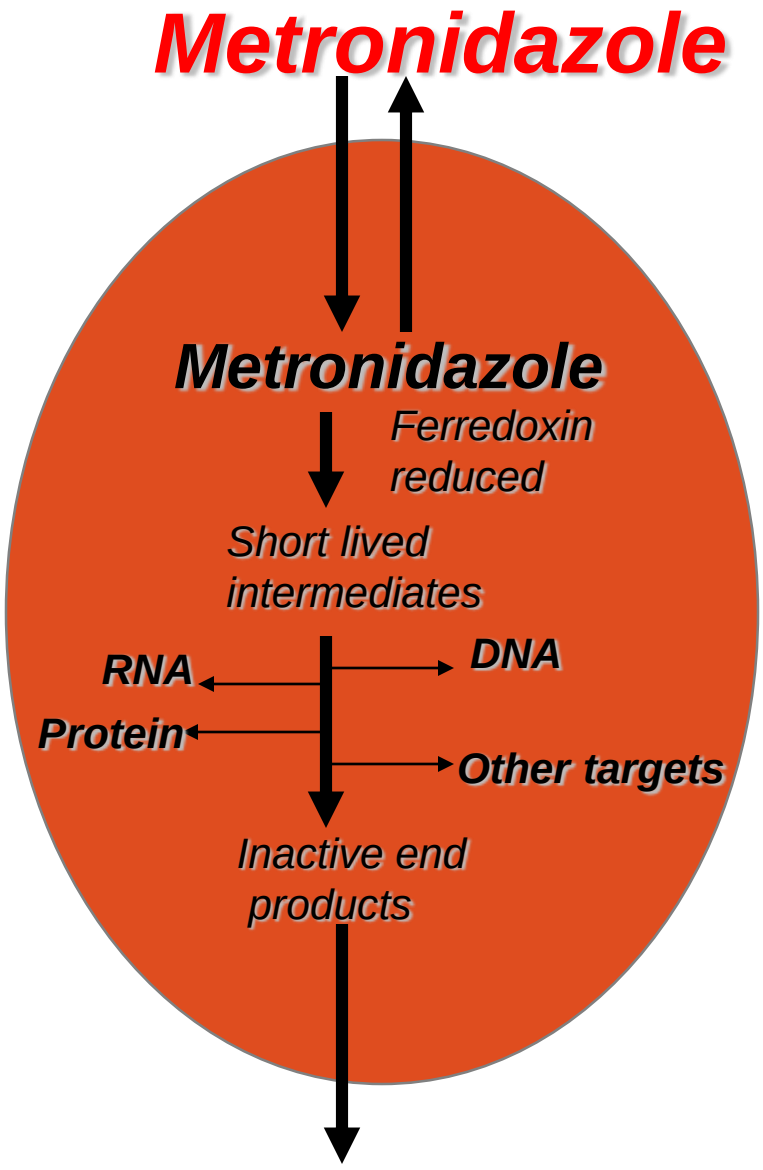
ANTIBIOTICS AFFECTING NUCLEIC ACID SYNTHESIS.

- **Fluoroquinolones**
- **Metronidazole**
- **Rifampin**

Rifampin

binds to RNA polymerase

- ✓ active against gram positive cocci
- ✓ bactericidal for *Mycobacterium*
- ✓ used for treatment and prevention of meningococcus



Mechanism of action of metronidazole on an anaerobic organism

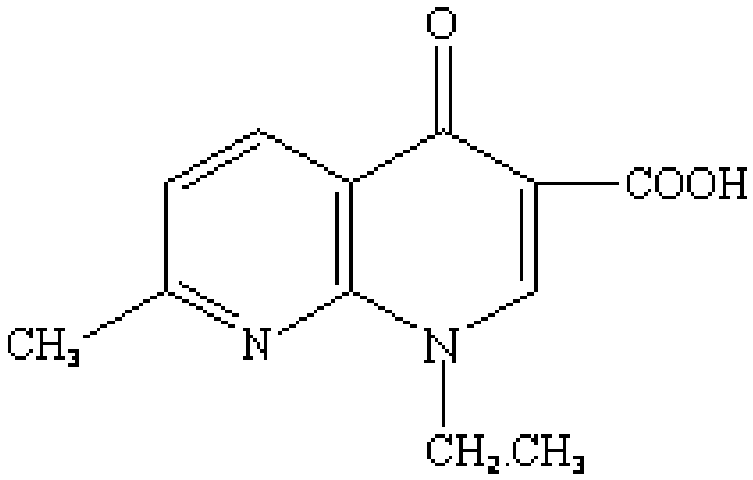
Metronidazole

- Mechanism of action:
  - Enters bacteria via cell diffusion
  - Activated via single reduction step by bacteria→ forms radicals → reacts with nucleic acid → cell death
- Spectrum of activity:
  - Anaerobic bacteria
  - Microaerophilic bacteria
  - Protozoa
- Resistance:
  - Rare
  - Mechanism: decreased activation (↓ redox reaction) of drug

Quinolones

Quinolones

- Parent drug: nalidixic acid



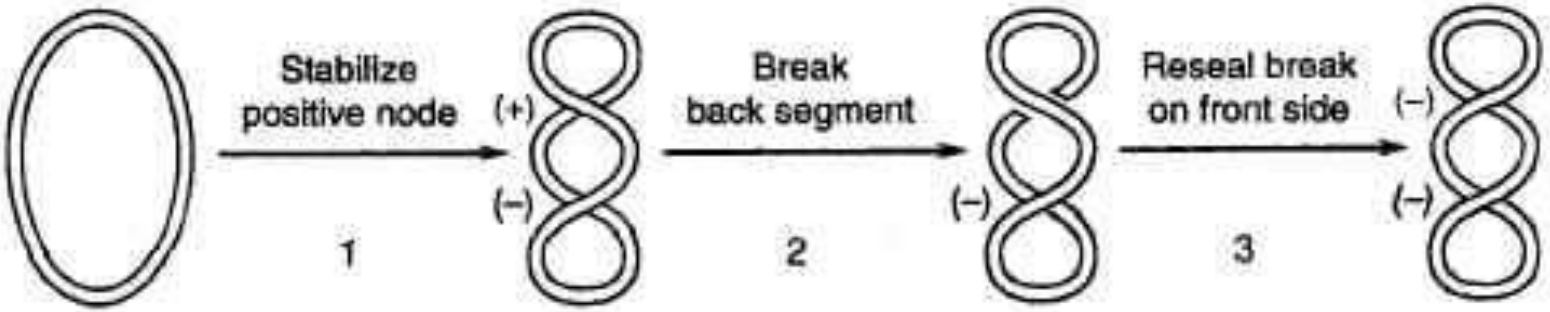
Classification

- Quinolones (1st generation)
  - Highly protein bound
  - Mostly used in UTIs
- Fluoroquinolones (2nd, 3rd and 4th generation)
  - Modified 1st generation quinolones
  - Not highly protein bound
  - Wide distribution to urine and other tissues; limited CSF penetration.

Mechanism of Action

- Dual MOA:
  1. Inhibition of bacterial DNA Gyrase (Topoisomerase II)
     1. Formation of quinolone-DNA-Gyrase complex
     2. Induced cleavage of DNA
  2. Inhibition of bacterial Topoisomerase IV
     1. Mechanism poorly understood

Mechanism of DNA Gyrase



Quinolones

- Drugs: norfloxacin, ciprofloxacin, ofloxacin, levofloxacin, moxifloxacin
- Mechanism of action:
  - Inhibit bacterial DNA synthesis by inhibiting DNA gyrase and topoisomerase IV → rapid cell death
  - Post antibiotic effect: lasts 1 to 2 hours, increases with increasing concentration
- Mechanism of resistance:
  - Chromosomal:
    - Alter target enzymes: DNA gyrase and topoisomerase IV
    - Decreased drug penetration: Pseudomonas, E. coli
  - Plasmid: seen in some K. pneumoniae and E. coli
  - Mutations in both target enzymes are needed to produce significant resistance

Antibiotic Resistance Cycle

Increased Antibiotic Use

Increase in resistant strains

Ineffective empiric therapy
➡ Increased morbidity
➡ More antibiotics

Increased hospitalization
➡ More antibiotics

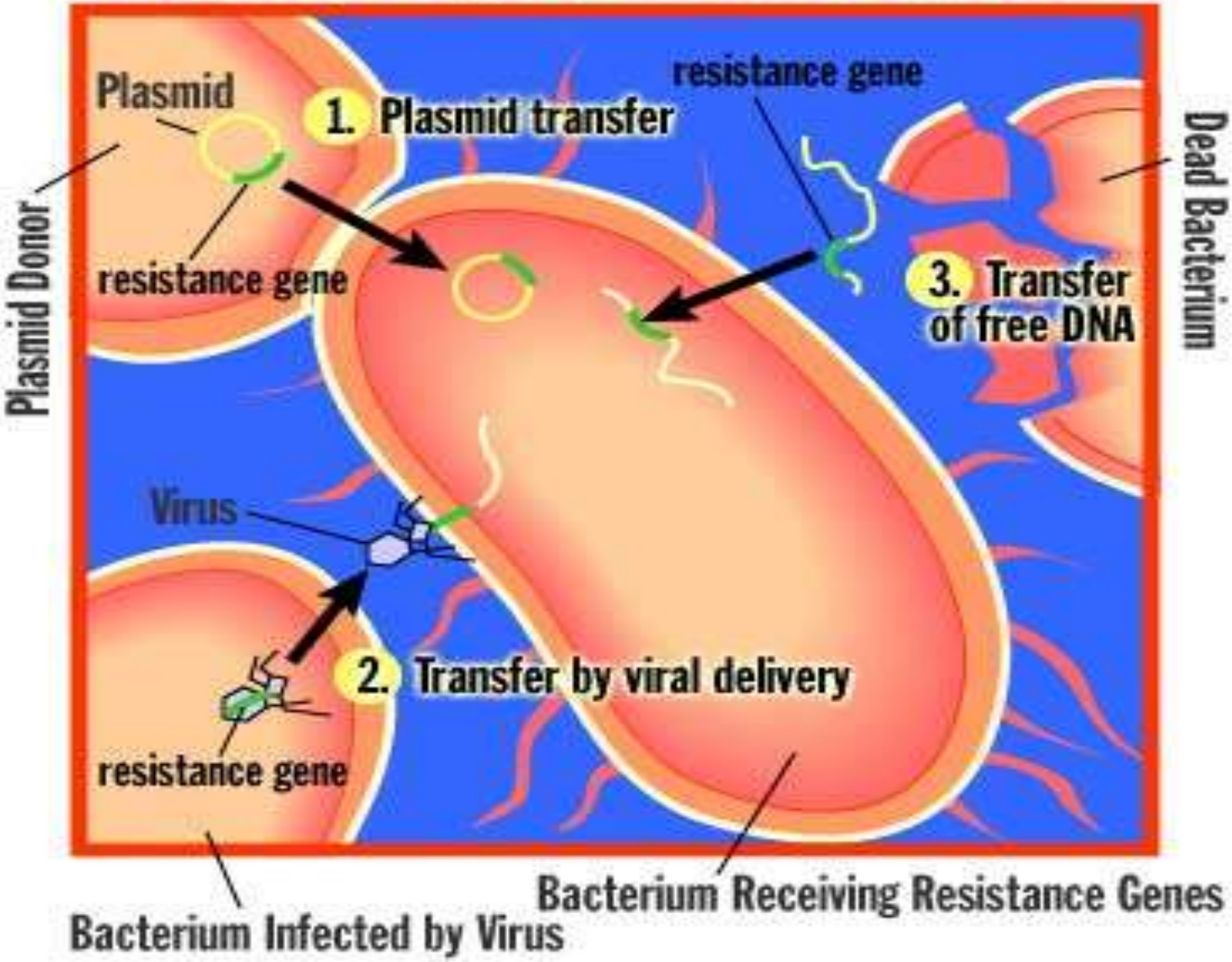
Increased healthcare resource use

Limited treatment alternatives
➡ More antibiotics
➡ Increased mortality

Resistance to antimicrobial drugs

- Natural
  - Chromosomal
- Acquired (mutation and genetic recombination)
  - Plasmid
  - Integron
  - Transposone

Transferring Resistance Genes



Gene Transfer Facilitates the Spread of Drug Resistance

Resistant and non-resistant bacteria exist

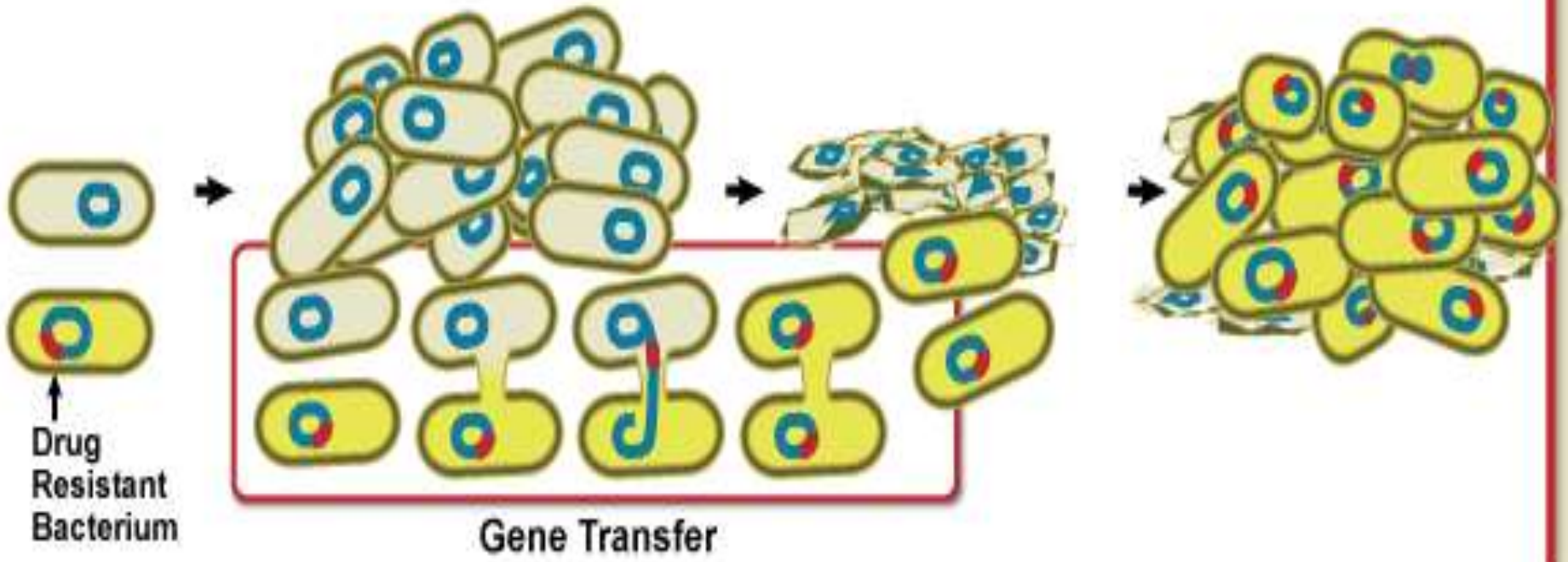
Bacterium multiply by the billions

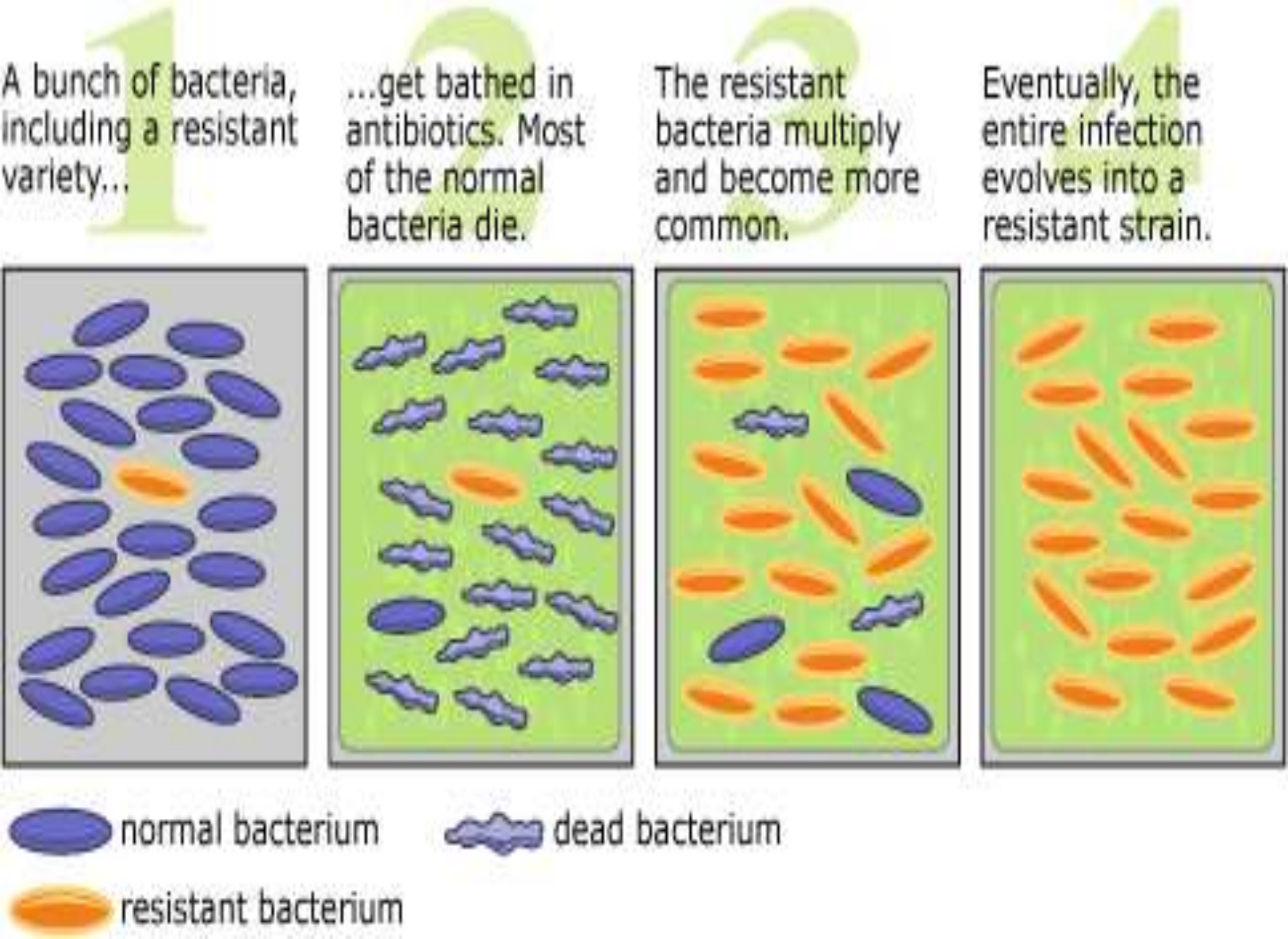
Bacteria that have drug resistant DNA may transfer a copy of these genes to other bacteria.

Non-resistant bacteria receive new DNA.

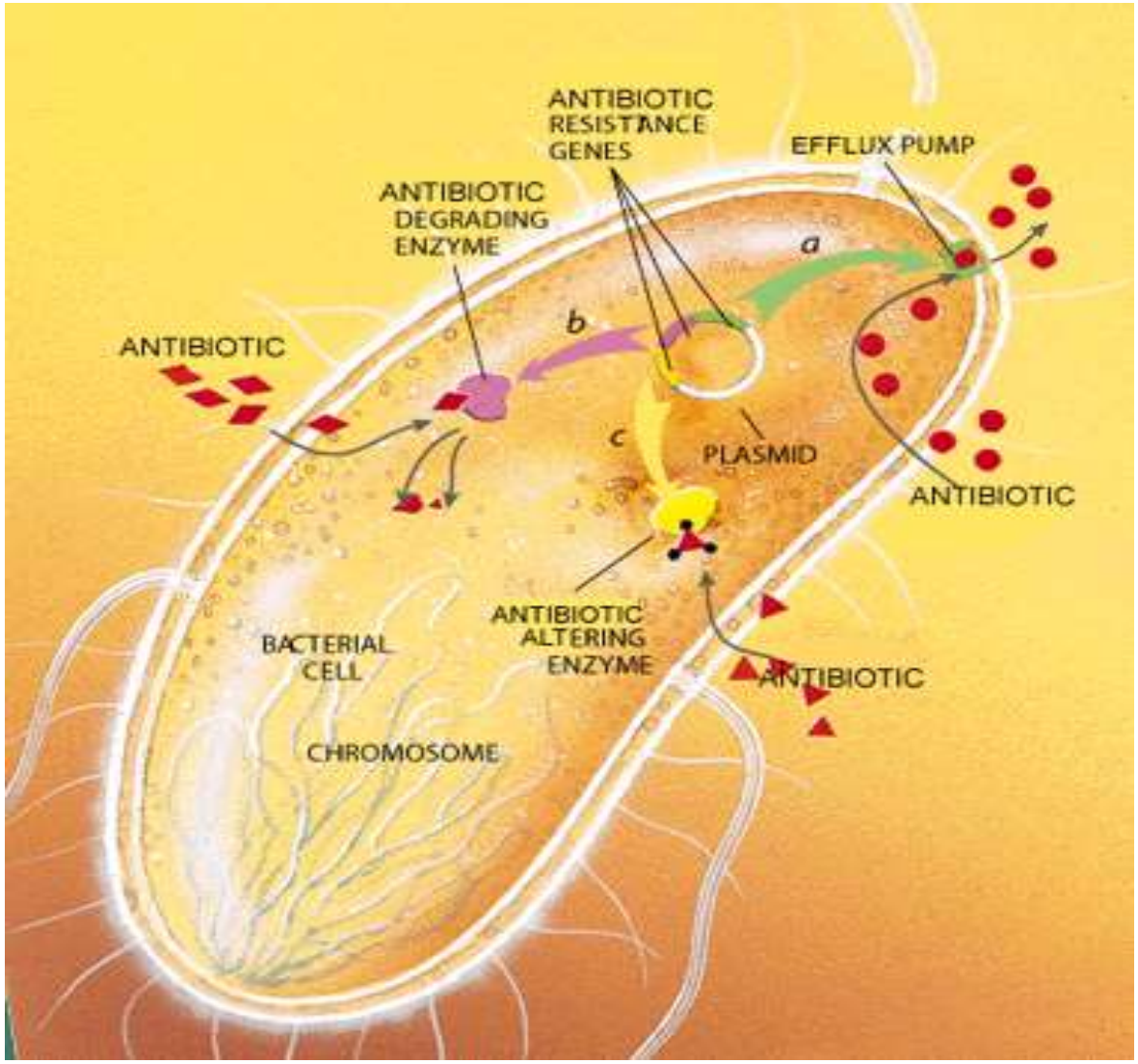
Non-resistant bacteria become resistant.
In the presence of drugs, only drug-resistant bacteria survive.

Drug resistant bacteria multiply and thrive.




A bunch of bacteria, including a resistant variety...
...get bathed in antibiotics. Most of the normal bacteria die.
The resistant bacteria multiply and become more common.
Eventually, the entire infection evolves into a resistant strain.
normal bacterium
dead bacterium
resistant bacterium

Resistance mechanisms



- Enzymatic degradation (beta-lactamases)
- Permeability changes (outer memrane protein)
- Target modification (Penicillin binding protein)
- Efflux pumps

Thank you for your attention!