MUNI
PHARM

INTRODUCTION TO CHEMOTHERAPY OF INFECTIONS
RESISTANCE TO ANTIBIOTICS

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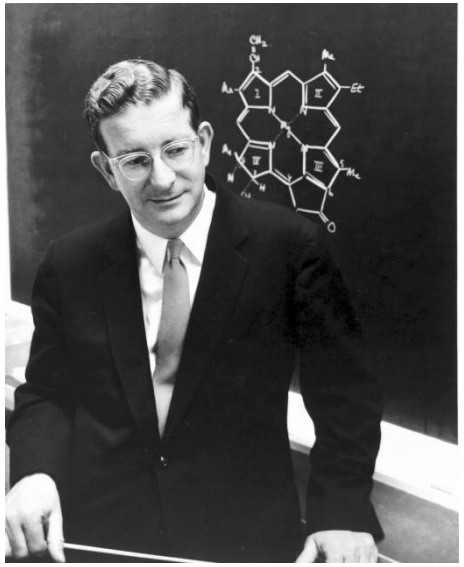
HISTORY

- **1929** Flemming discovered penicillin
- **1935** first sulfonamides synthetised
- **1939** Penicillin was isolated from *Penicillium notatum* by Florey a Chain
- **1940** Woodward determines the chemical structure of penicillin



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Bacteria

Size of the most cells:

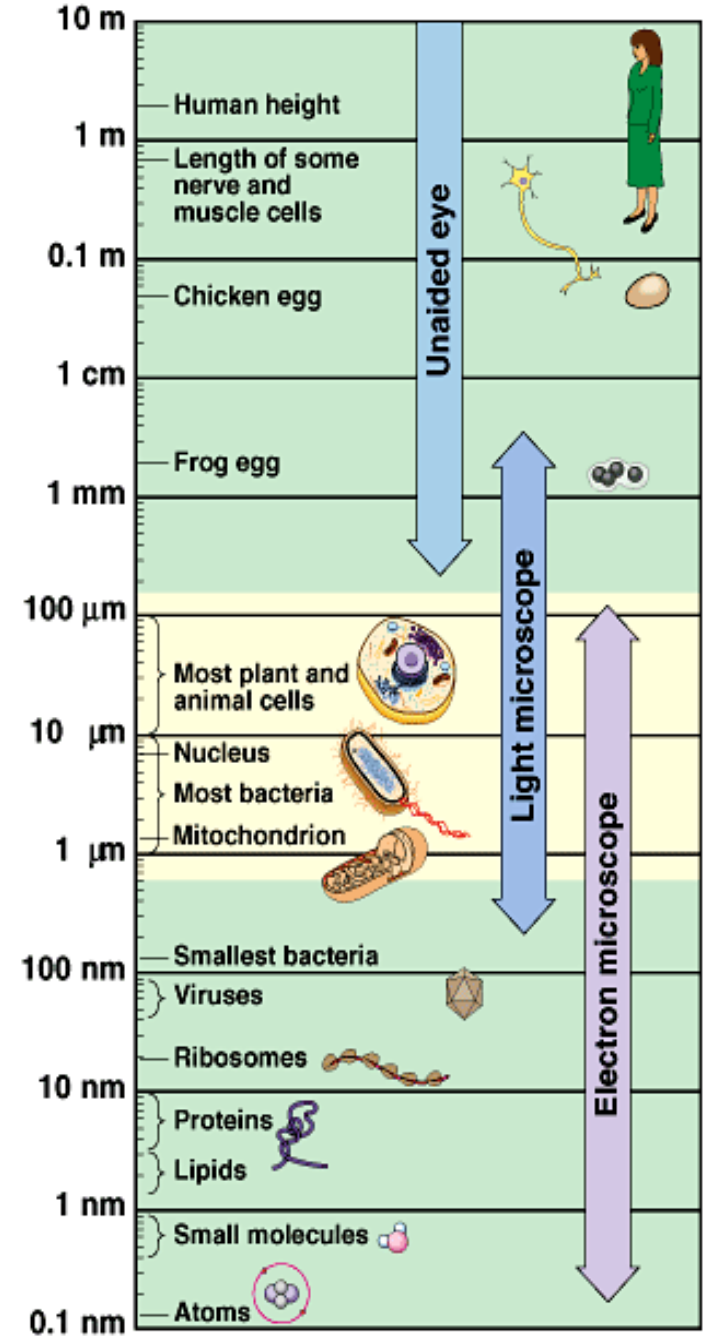
1µm – 100µm

– *Prokaryotes*

usually in range 1µm - 10µm

– *Eukaryotes*

usually in range 10µm - 100µm



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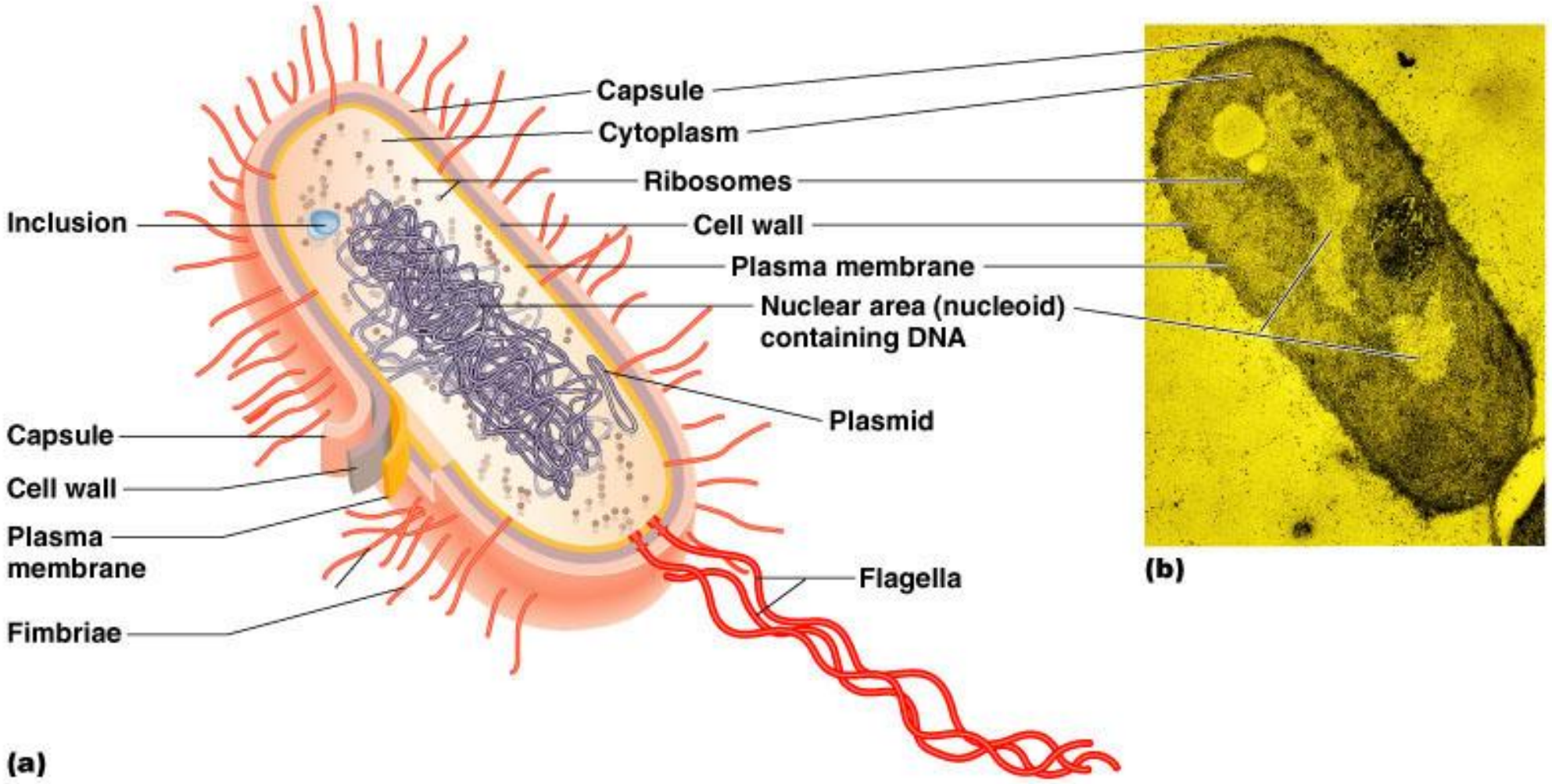
Bacterial cell



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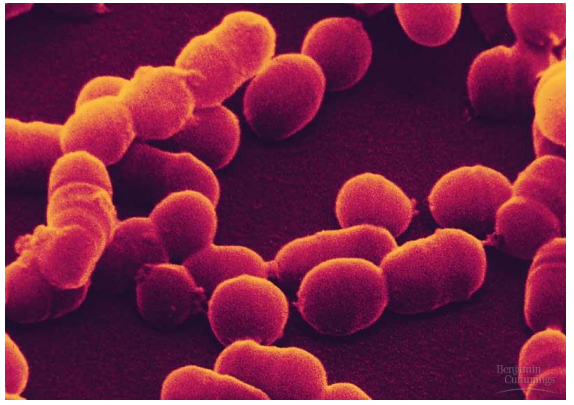
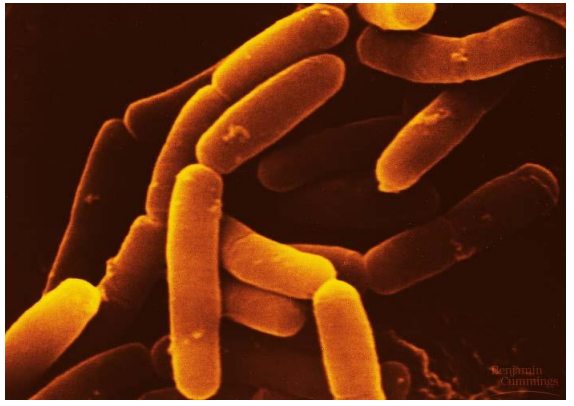
Basic characteristics (I.)

- According to their shape bacteria are divided on classes:

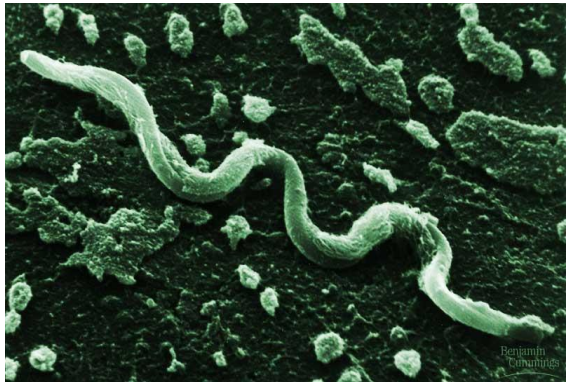
coccus



bacillus



spirillum



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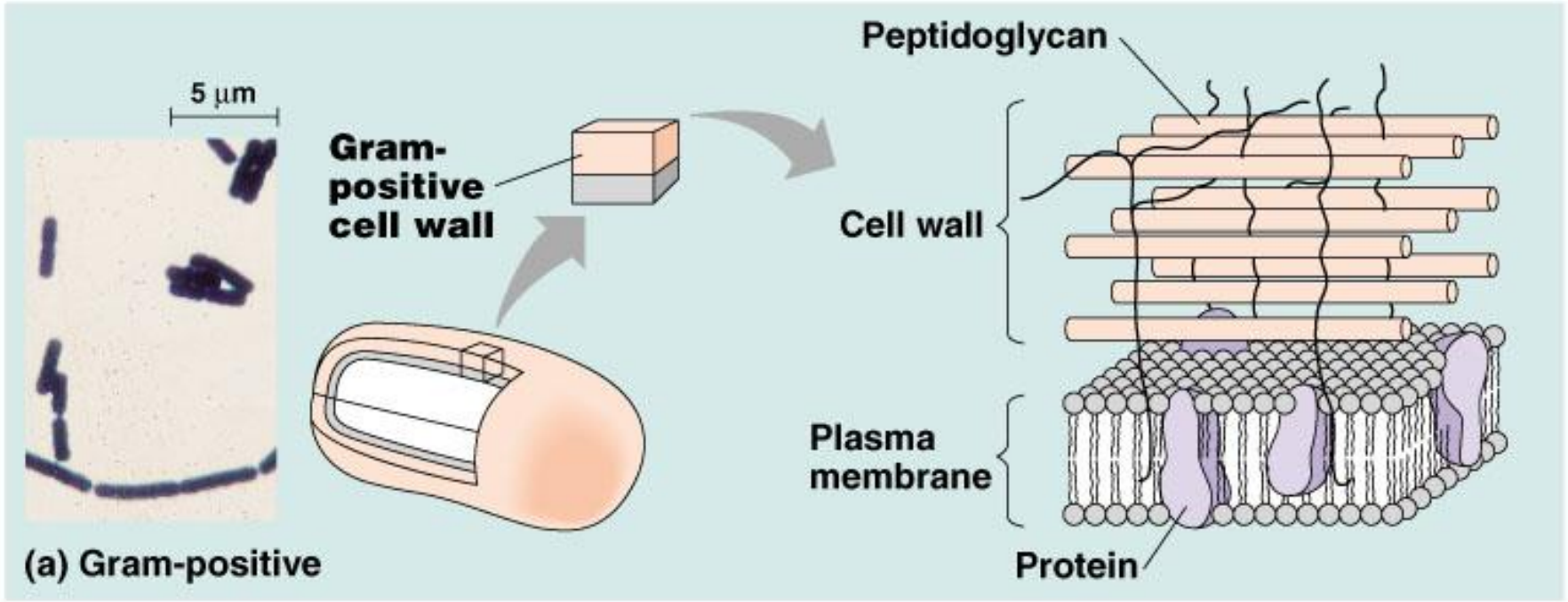
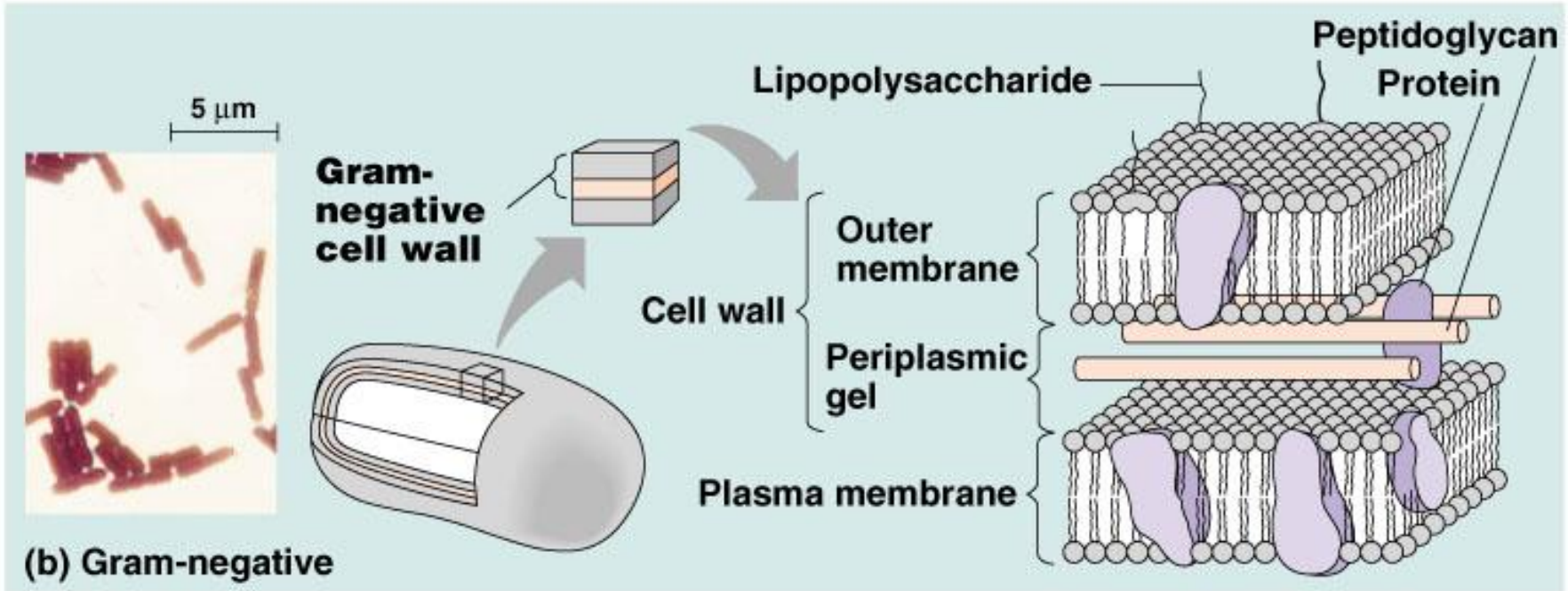
Basic characteristics (II.)

Based on architecture of bacterial wall (by Gram staining):

- Gram-positive bacteria
  - high amount of peptidoglycan in the cell wall
  - stained dark blue or violet by Gram staining
- Gram-negative bacteria
  - thin peptidoglycan layer is placed between two PM
  - stained red or pink color by Gram staining
  - generally more dangerous to humans and more resistant to ATBs





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Principles of ATB Therapy (I.)

1. Bacteriostatic vs. bactericidal agents:

- *Static* – restrict the spread of infection → immune system kills pathogens
- ATB can be *static* for one and *cidal* for other microbe type (CHP – *static* against G- and *cidal* for e.g. *S. pneumoniae*)
- Bacteriostatic ATB can act bactericidal in a higher conc.

2. Minimum inhibitory concentration (MIC): lowest conc. of ATB that inhibits visible growth of a microorganism after overnight incubation. Effective treatment = conc. ATB higher than MIC (2-5x)

3. Minimum bactericidal concentration (MBC): lowest concentration of antibiotic required to kill the germ

Principles of ATB Therapy (II.)

- **Concentration-Dependent Killing**
  = rate and extent of ATB is more a function of conc. than of time, with killing most closely related to the peak concentrations achieved (AG, F QUIN)

Time-Dependent Killing
= effect is dependent on the time during which ATB concentration at site of infection is above MIC (beta-lactams, glycopeptides, MAK, clindamycin)

Post-Antibiotic Effect
= continued suppression of antibacterial growth after the administration of ATB has ceased and serum conc. have fallen below MIC (AG have the longest)

ANTIBIOTIC RESISTANCE

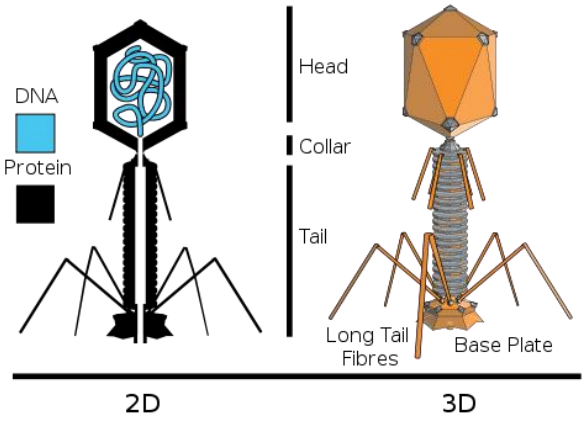
- **Primary** – genetically determined insensitivity of bacteria to ATB (w/o former contact with ATB)

- **Secondary** – during or following therapy, selection of resistant strains in the bacterial population

  1) genotype

  - chromosomal mutation (spontaneous, independent on ATB)
  - by transport (**plasmids**):
    a) **transduction** (bacteriophage)
    b) **conjugation** (pillus)

  2) phenotype – adaptation



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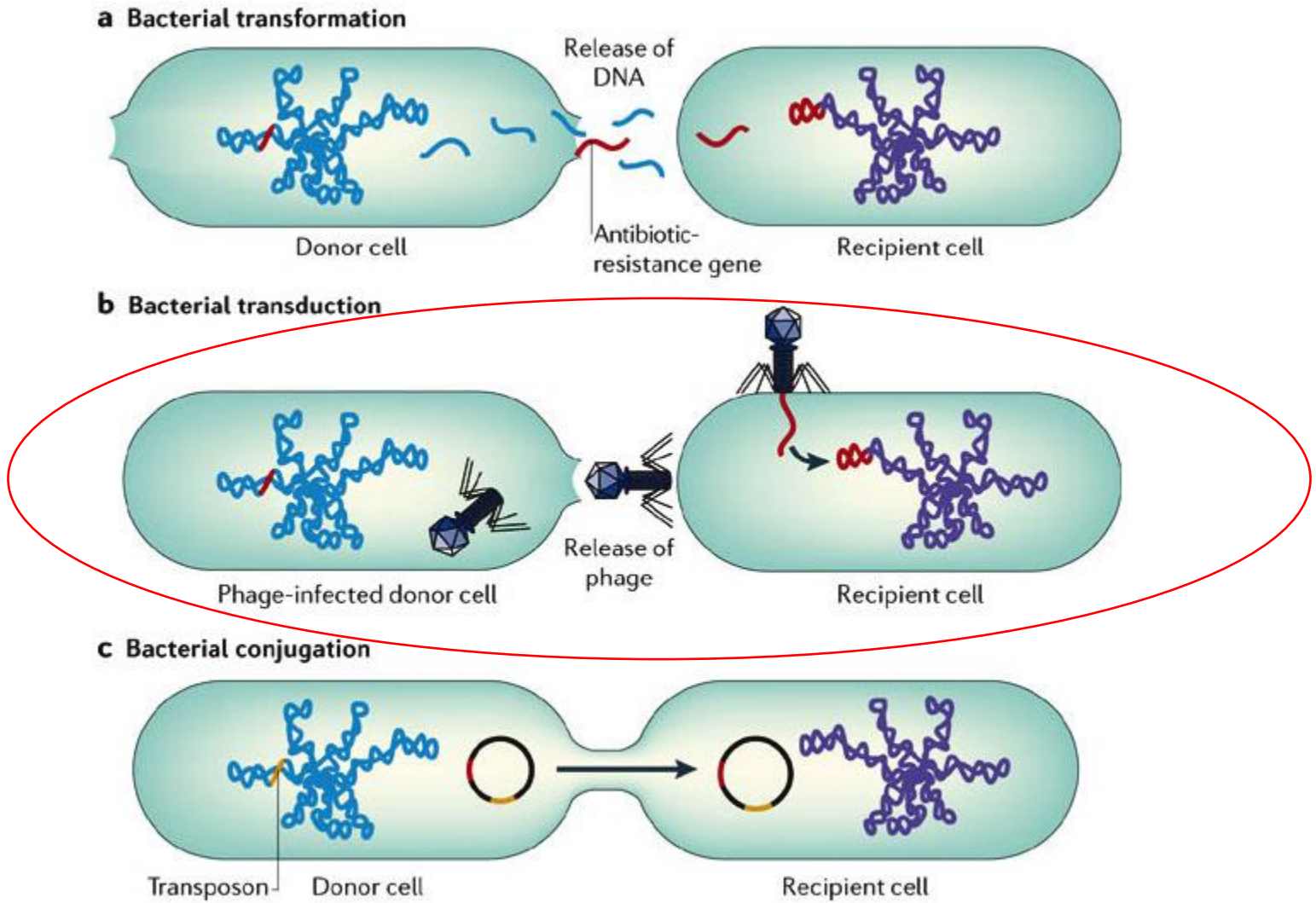
Transduction



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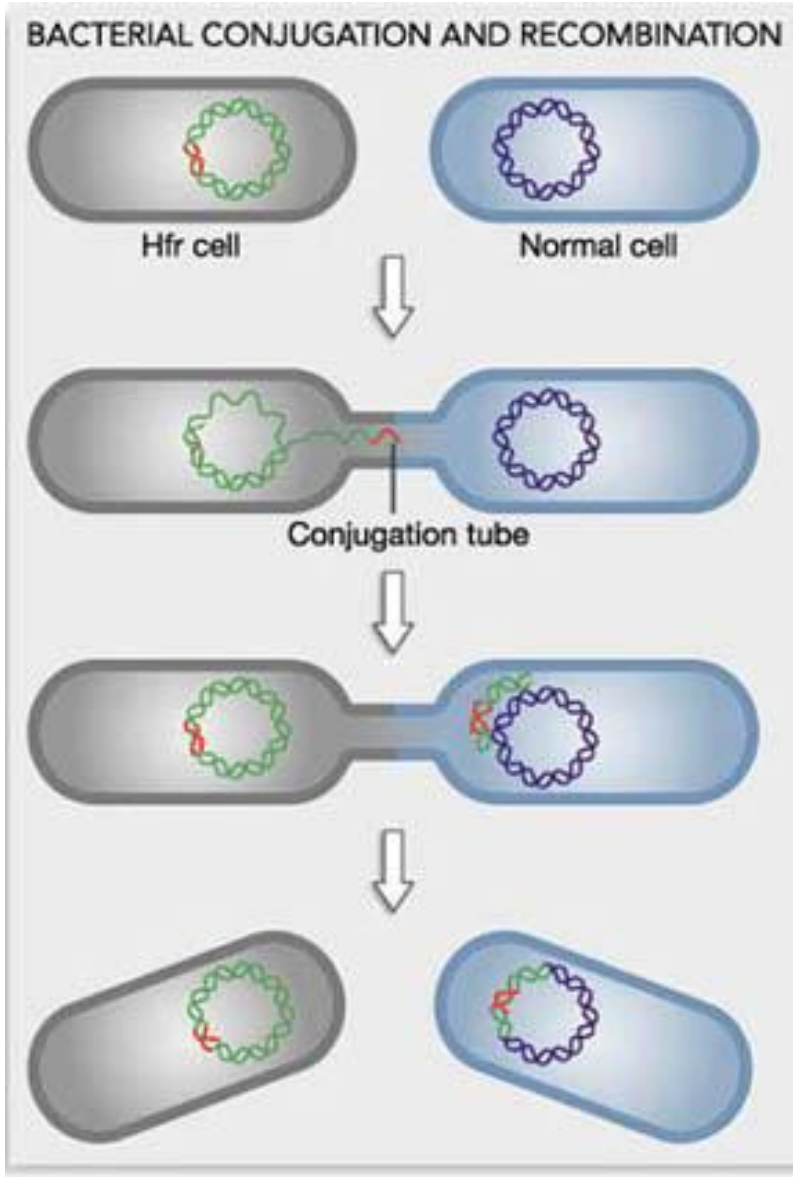
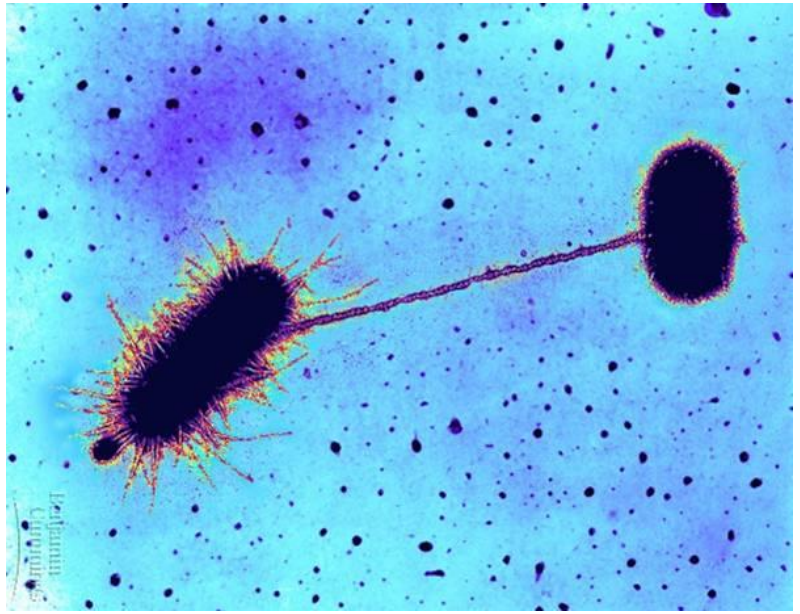
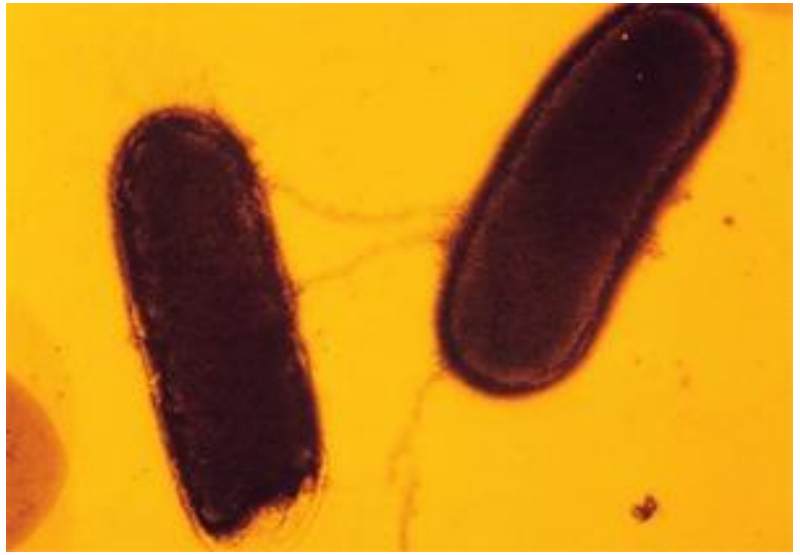
Bacterial Conjugation







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Types of Resistance

- **Cross-resistance** -- simple mechanism of action -- chemically or MoA similar ATB

- **Multiple resistance** -- of multiple mechanisms -- unrelated antibiotics

Mechanisms of Resistance

- Change of permeability of bacterial packaging

  Influx change

  -Gram negative bacteria

  Eflux change (increased excretion)

  -Tetracyclines

- Inactivation

  Beta-lactamases

  Chloramphenicol acetyl transferase

Mechanisms of Resistance

- Change of the target site

  Penicillin binding proteins (PEN)

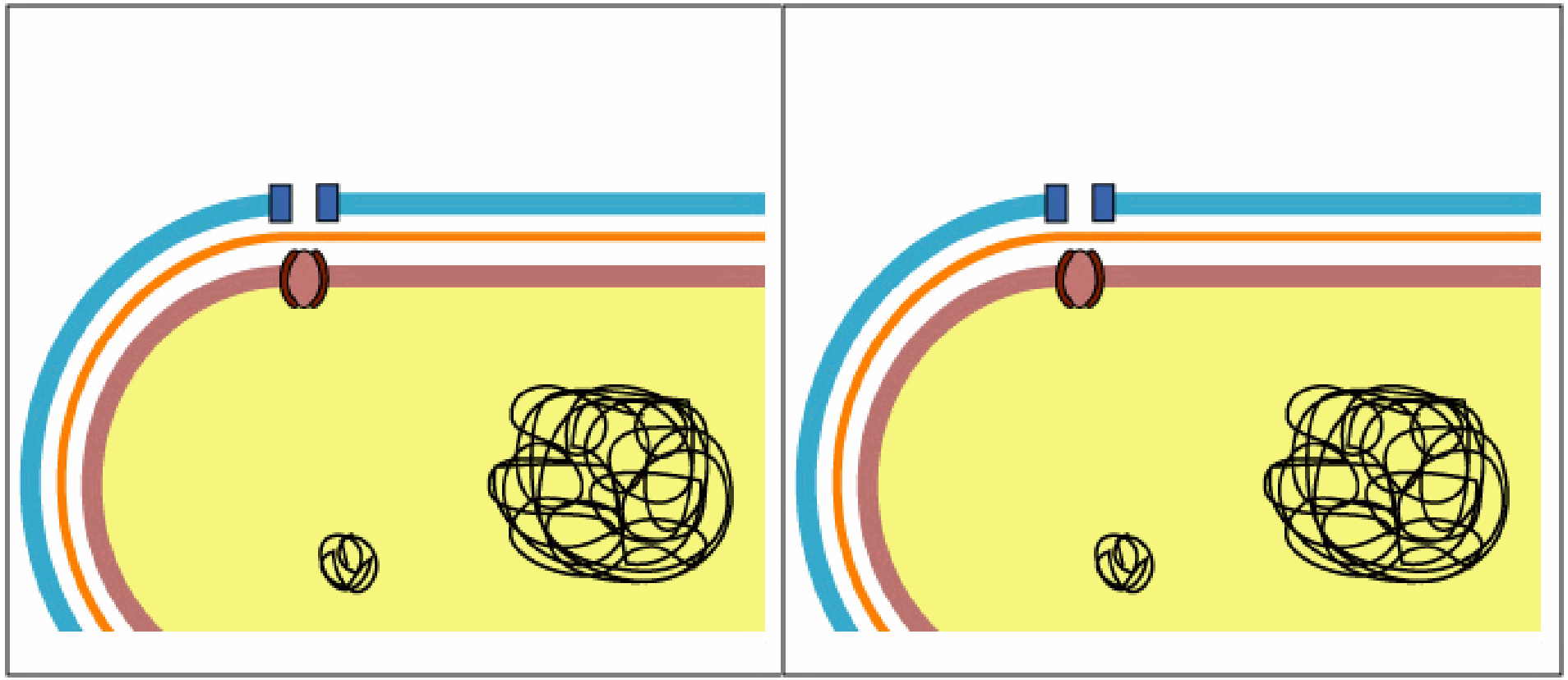
  30S ribosomal subunit (streptomycin)

- Replace of sensitive metabolic pathway

  (target is bypassed by new MTBlite)

  Acquisition of resistant enzyme (SA, trimethoprim)

Influx change



Microbe Library

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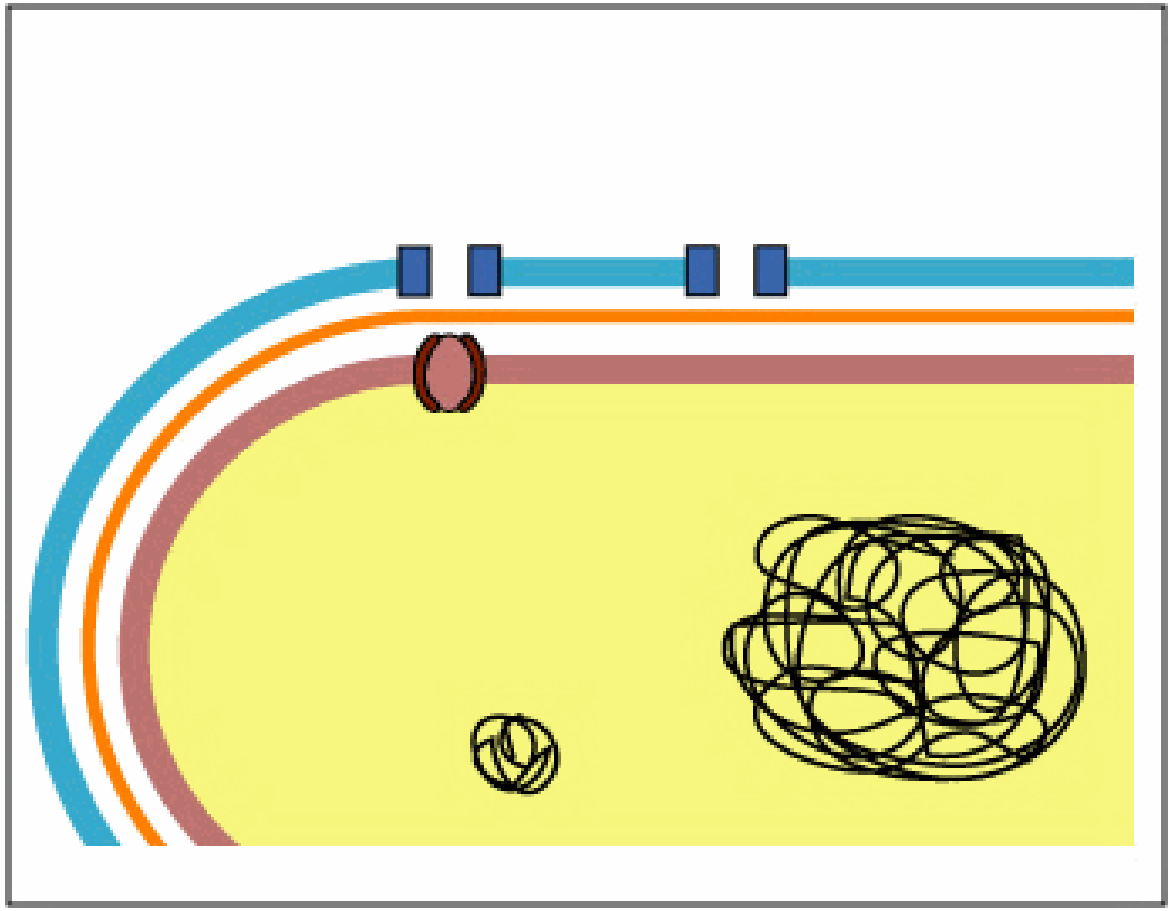
Eflux change



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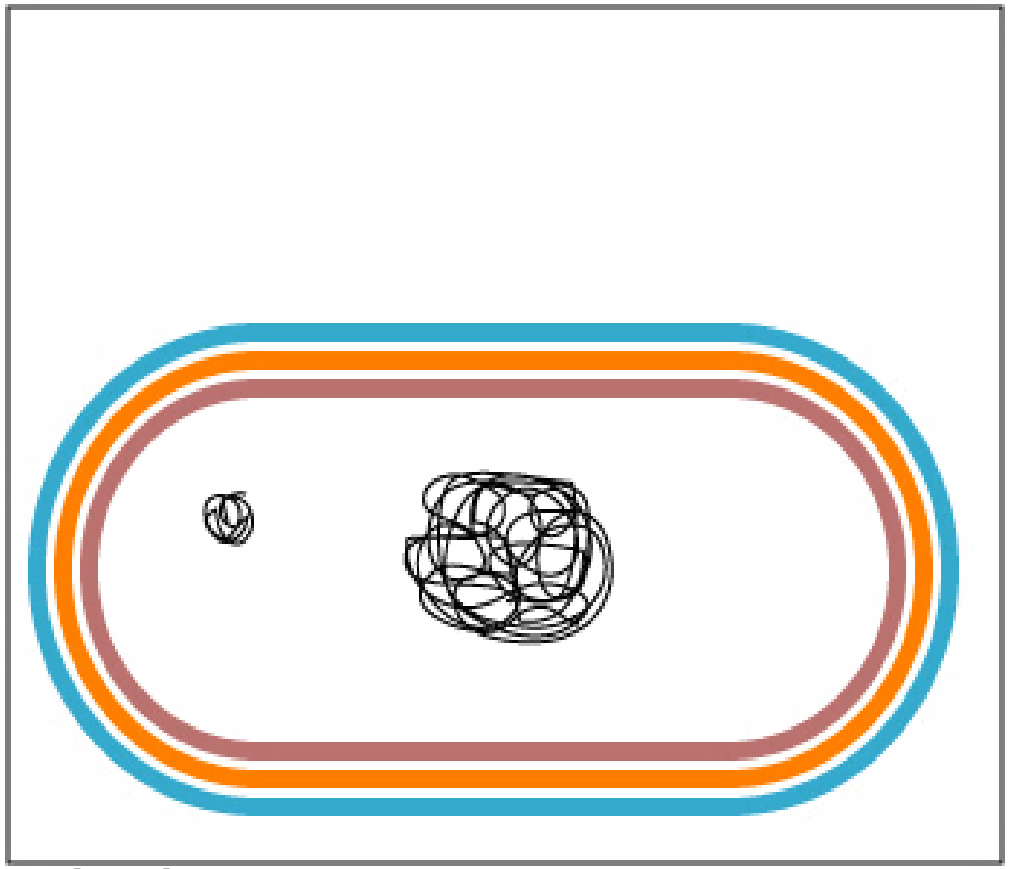
Inactivation



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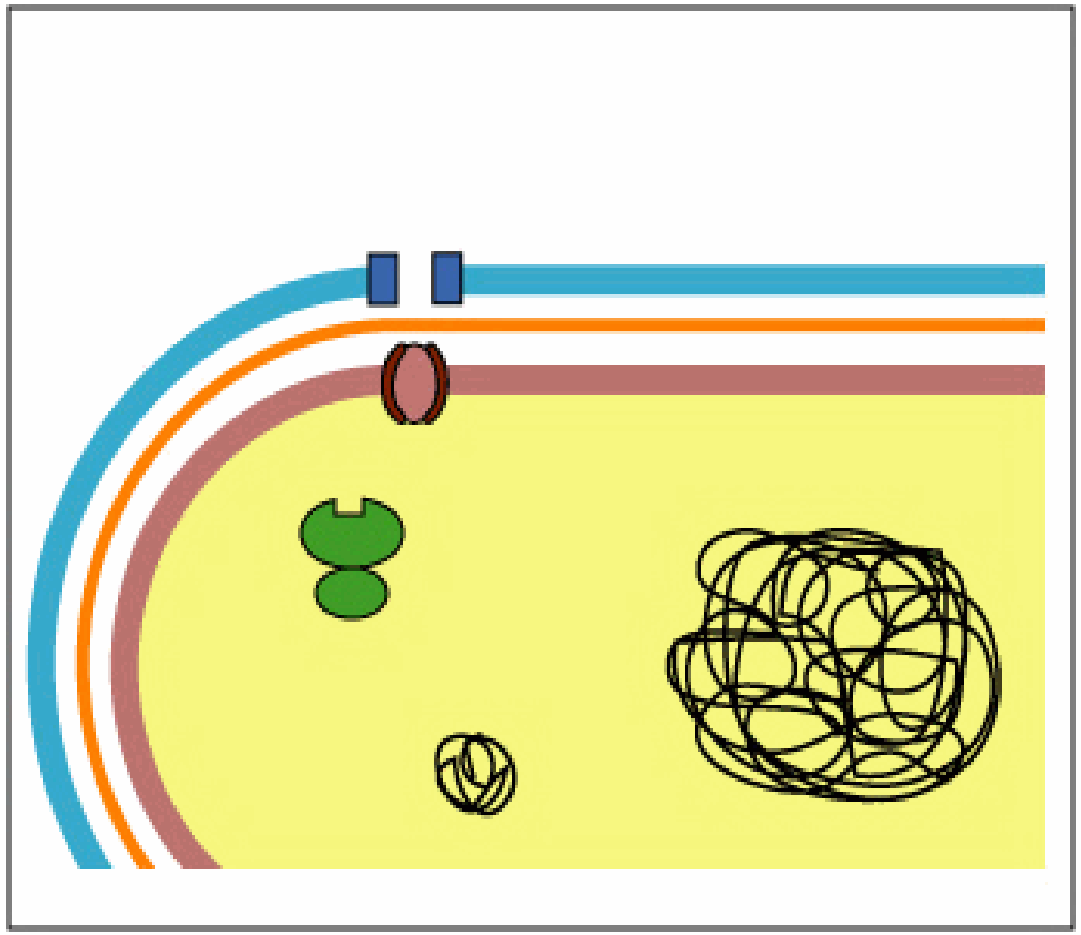
Change of the target site



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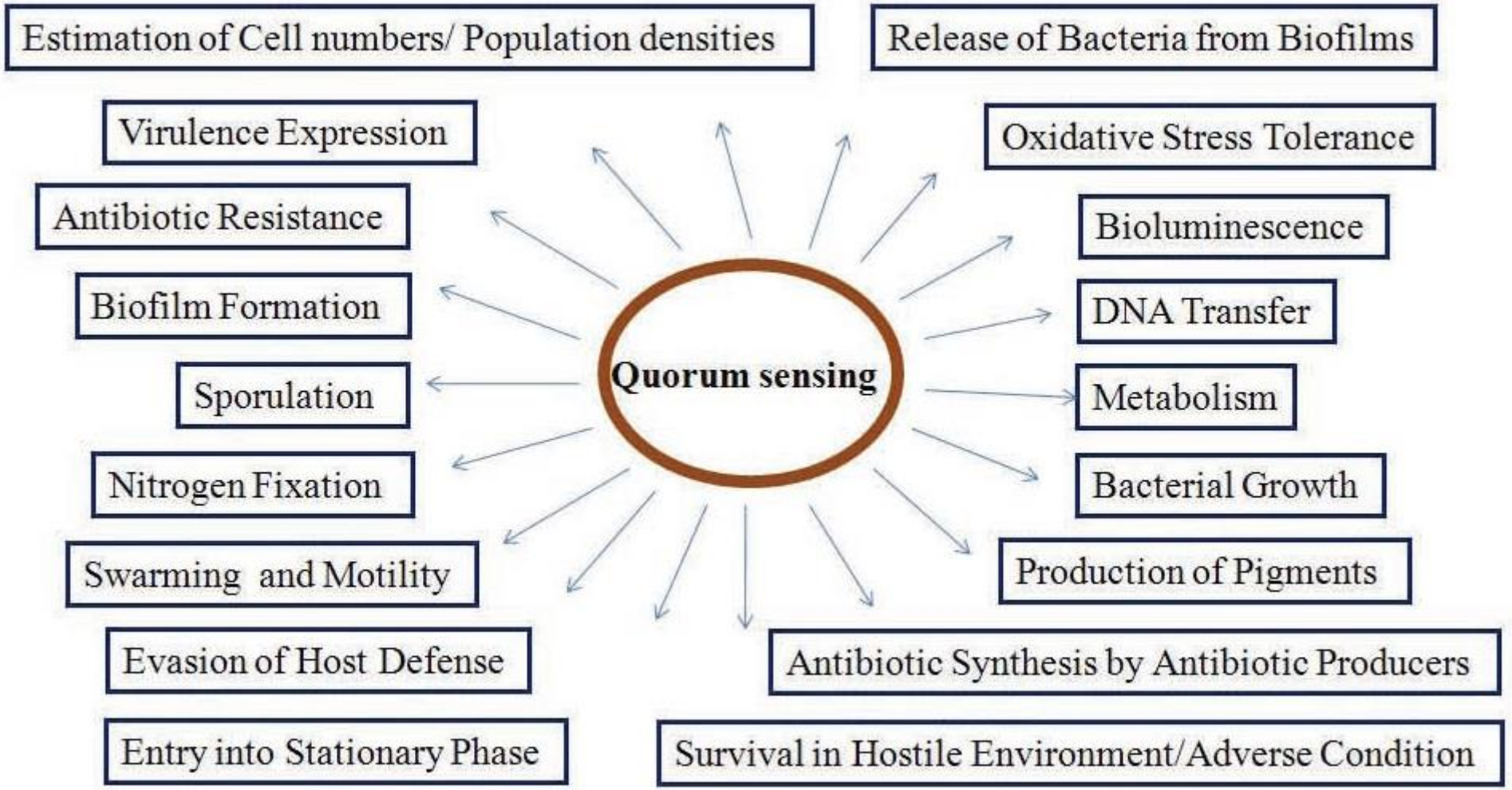
Physiological adaptation
Quorum sensing

Quorum sensing - bacterial signaling:

- Low molecular weight compounds
- Inhibit macrophage & T lymphocyte functioning
- Able to determine cell density- switch on/off genes
- Increase density to an effective infectivity dose without alerting host of impending attack; pathogenesis genes not switched on.



Bhardwaj et al.: Recent Patents on Anti-Infective Drug Discovery, 2013, Vol. 8, No. 1

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Side Effects of ATB (I.)

- **Alergic reaction:**

Symptoms: urticaria, exanthema, contact dermatitis, drug fever, vascular symptoms, anaphylactic shock

- **Toxic reaction:**

1) Local irritation:

Symptoms: painful application (i.m.), trombophlebitis (i.v.), GIT disorders (p.o.)

2) Nephrotoxic effects:

AG, Polymyxin, Colistin, Neomycin, Vancomycin

Symptoms: proteinuria, haematuria, necrosis of renal tubules, renal failure

Side Effects of ATB (II.)

3) Hepatotoxic effects:

Symptoms: elevation of liver enzymes

Oxacilin, Cotrimoxasol, SA, Ery, Rif, Nitrofurantoin

4) Haematotoxic effects:

Symptoms: hematopoiesis impairment (aplastic anemia, agranulocytosis), HA

CHP – Reversible and irreversible decreased hematopoiesis !!!

Side Effects of ATB (III.)

5) Ototoxic effects:

Depends on ATB conc., even 1 dose is dangerous!!

Higher risk in reduced renal function, and parallel administration of furosemide.

AG (Streptomycin, Gentamycin)

6) Neurotoxic effects:

Neuromuscular blockade (bound on receptors of synaptic signal transport).

Neuropathy: **Nitrofur., Vancomycin, Polymyxin, Colistin**

ATB and Pregnancy

1. *w/o restriction (cave anaphylaxis!):*

   PEN, CEP, MAC, LIN, imipenem, aztreonam

2. *CI in the I. trimester:*

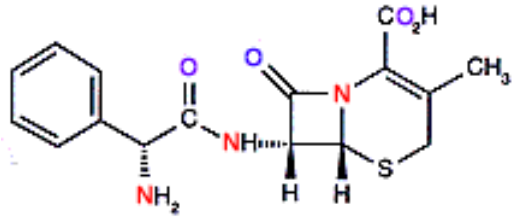
   CHP(+III.), SA (+III.), nonF QUIN, trimetoprim (+III.), nitrofurantoin, rifampicin

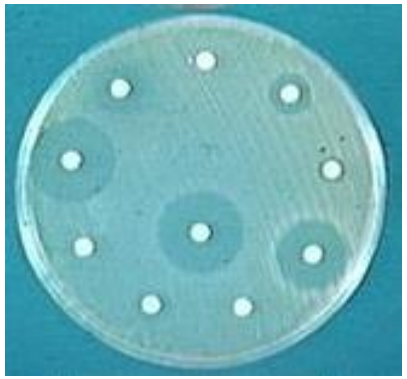
3. *CI during whole pregnancy:*

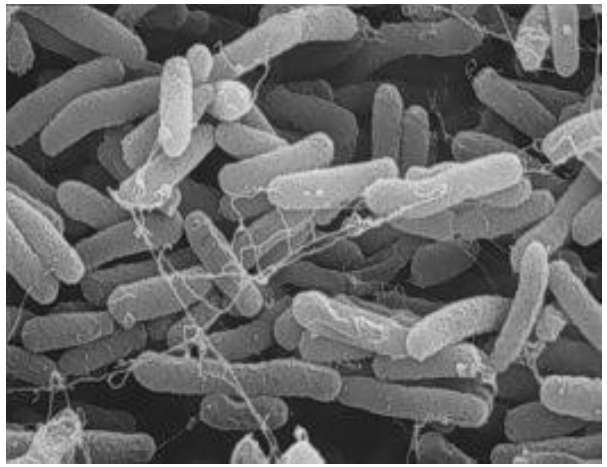
   AG, TET, F QUIN, vankomycin, colistin

Classification of ATB

- Chemical structure
- Spectrum of effect
  - Narrow-spectrum
  - Extended-spectrum
  - Broad-spectrum
- Effect on bacteria
  - bacteriostatic
  - bactericidal
- Mechanism of action







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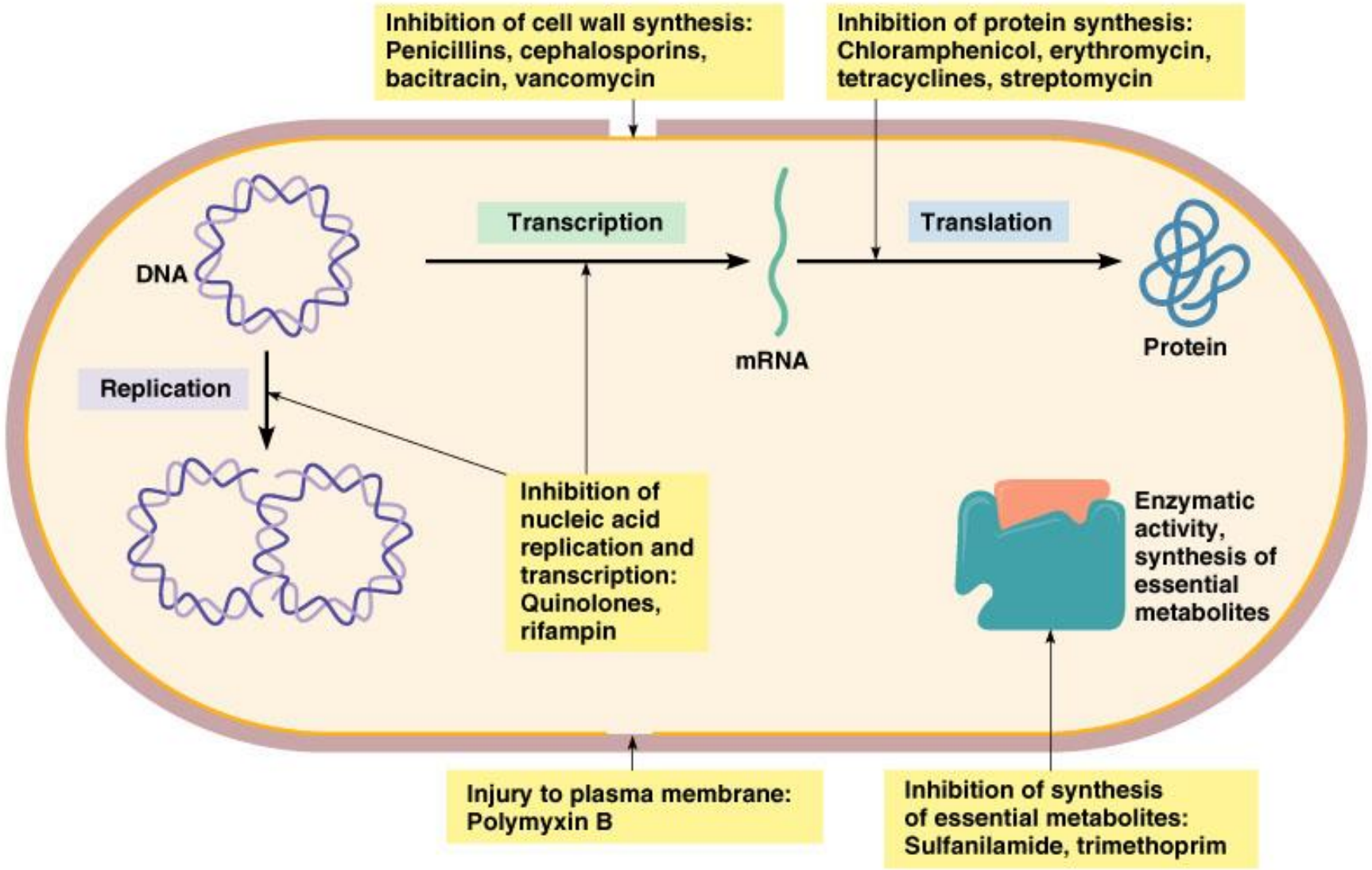
Mechanisms of ATB action



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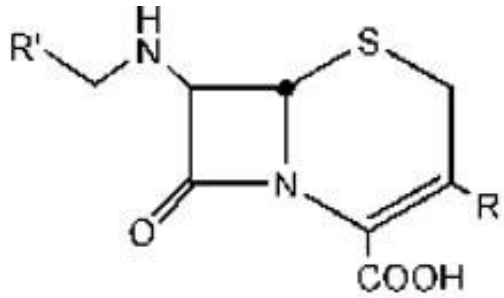
Classes of ATB

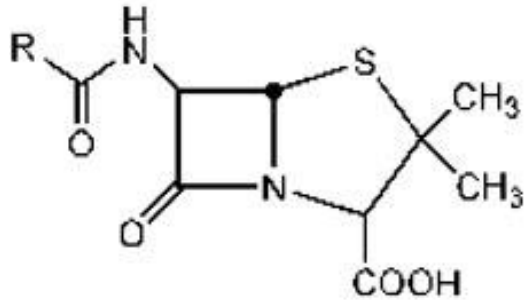
1. **Beta-lactam ATB**
   - Penicillins
   - Cephalosporins
   - Monobactams
   - Carbapenems
2. **Aminoglycosides**
3. **Tetracyclines**
4. **Amphenicols**
5. **Macrolides**
6. **Lincosamides**
7. **Ansamycins**
8. **Peptides**
   - Polypeptides
   - Glycopeptides
6. **Sulfonamides**
7. **Pyrimidines**
8. **Quinolones**
9. **AntiTBC, antiLPR**

BETA-LACTAM ATB

- Bactericidal
- Low toxicity
- Good tolerance
- The largest group
- Administration – i.v., p.o.
- Frequent occurrence of allergic reactions
- Resistance: Beta-lactamases cleave beta-lactam ring



Cephalosporin



Penicillin

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Penicillins

- **Basic PNC**

  penG (benzylPNC), penV (fenoxymethylPNC)

- **Against staphylococus PNC**

  oxacillin, cloxacillin, dicloxacillin

- **Broad-spectrum PNC**

  - Aminopenicillins (ampicillin, amoxicillin)
  - Carboxypenicilins (ticarcillin)
  - Ureidopenicilins (piperacillin)

} „Anti-Pseudomonal“

Mechanism of action

1. Bound on „*penicillin binding proteins*“ (PBPs) in bacterial wall

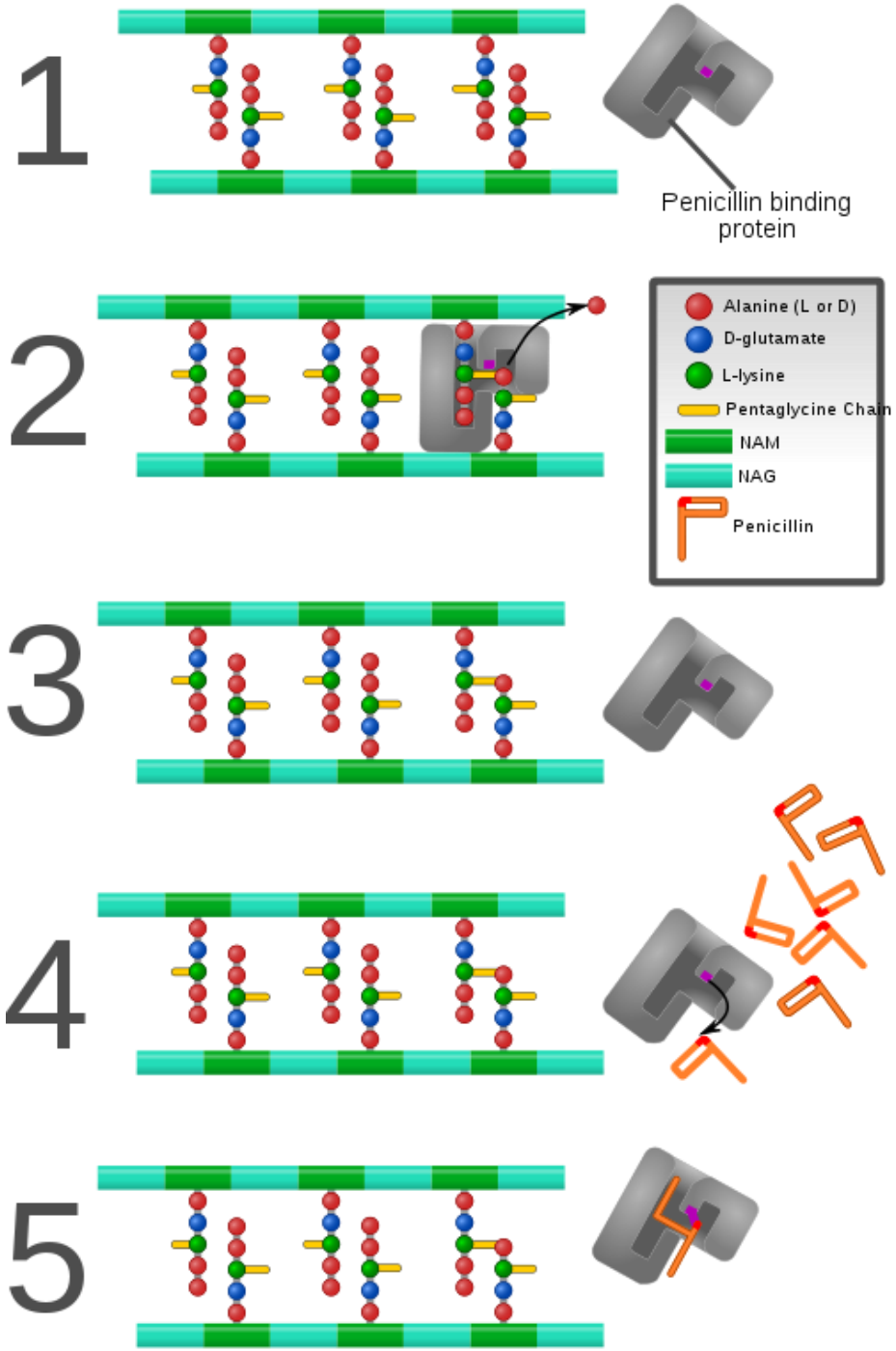
   (acylation = inactivation)

2. Inhibition of transpeptidases

   (inhibition of peptidoglycan stabilization in the wall by cross links)

3. Lysis of bacterial cell

   (autolysins = bacterial own enzymes)


1
Penicillin binding protein
2
Alanine (L or D)
D-glutamate
L-lysine
Pentaglycine Chain
NAM
NAG
Penicillin
3
4
5

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Combination of Penicillins

1. ***Inhibitors of beta-lactamases***

   - Clavulanate

   - Sulbactam

   - Tazobactam

   (Responsible for frequent GI disorders)

2. ***Aminoglycosides***

   = synergic effect of combination (PNC change wall permeability and facilitate penetration of AG). *CAVE* incompatibility (inactive complexes formation in combined infusion)

Cephalosporins

- **I. Generation** (G+, *E. coli*, *Klebsiella*, *Neisserie* …)
  Cefalotin, Cefazolin, Cefalexin (p.o.), Cefadroxil (p.o.)

- **II. Generation** (weak G+, more G- incl. *H. influenzae*)
  Cefoxitin, Cefuroxim, Cefuroxim axetil (p.o.)

- **III. Generation** (hl. G- incl. entrobacteria, the weakest to G+)
  Cefotaxim, Ceftriaxon (DofCh in meningitis), Ceftazidim (*PE*)

- **IV. Generation** (highly effective against G+ i G- incl. *Pseudomonas aeruginosa*)
  Cefepim, Cefpirom

Monobactams

- **Aztreonam**
  - Effect mainly on G- incl. resistant strains *P.aeruginosa*, *Enterobactericeae*
  - No effect on G+ and anaerobes
  - Combination with other ATB effective on G+ (non-toxic alternative to AG – sepsis, CNS, airways …)
  - Good penetration into the inflammatory tissues, parenteral application

Carbapenems

- **Imipenem** (+cilastatin = inhibitor of dehydropeptidases → blocks degradation in kidney)
- **Meropenem**
  - Effect against pencillinase-producing G+ and G-, anaerobes, and *P. aeruginosa*
  - Resistant against majority of beta-lactamases
  - Maximal wide range of antimicrobial effect
  - Reserve !!! For the treatment of severe, polymicrobial or multiresistant infections

…to be continued

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