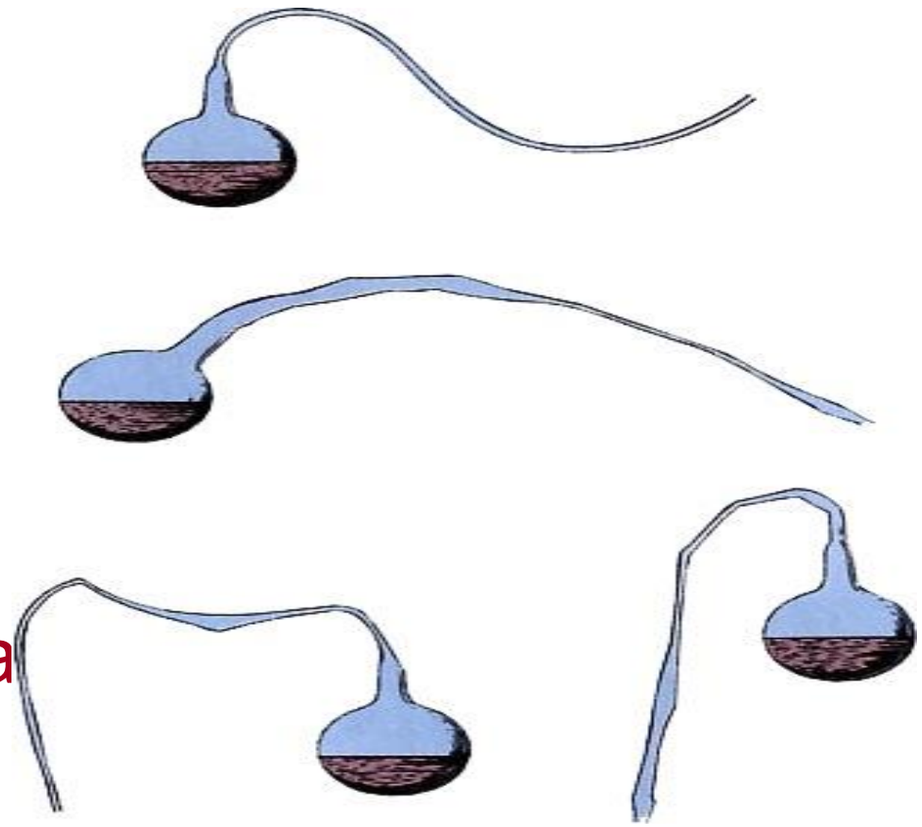
20.201
Bacteria, Antibiotics, and Antibiotic Therapy

October 30, 2013

History of Microbiolgy

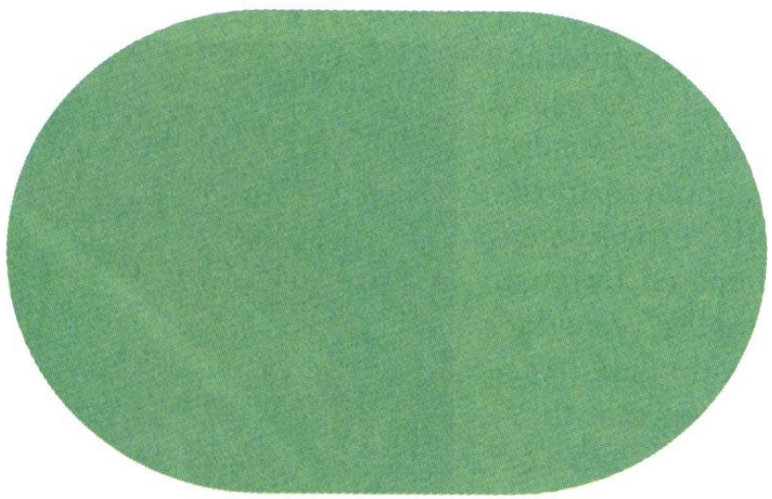
- Spontaneous Generation
  - Aristotle 384-322 B.C.
- Example of maggots arising from spoiled meat
  - Francisco Redi 1626-1697
- Air carried spores that led to microbial growth
  - Louis Pasteur 1822-1895
  - Pasteurization
  - Vaccines for anthrax and rabies



Source: Prescott, Lansing, John Harley, and Donald Klein. *Microbiology*. 4th ed. McGraw-Hill, 1999.

Microbiology, 4th Ed., Prescott

Microbes: In Perspective

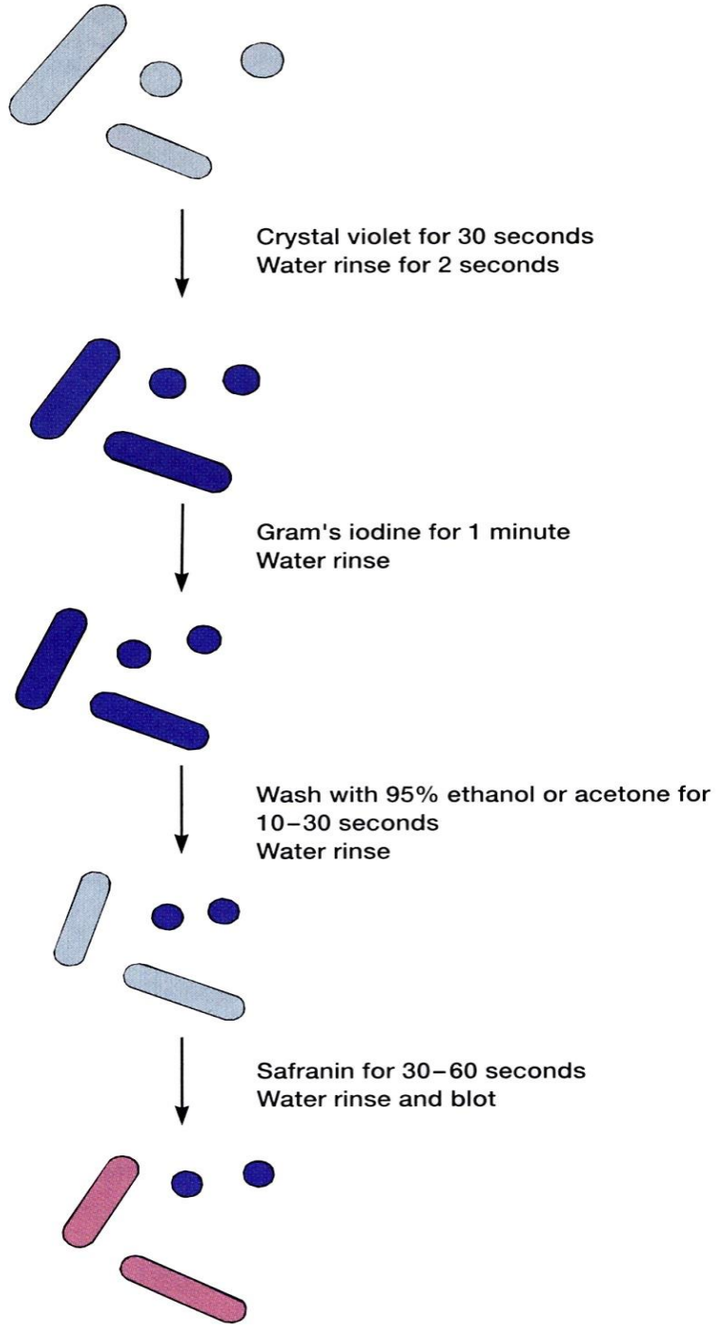
	diameter or l x w (nm)	
Red blood cell	7,000	of *E. coli* below
E. coli	1,300 × 4,000	
Rickettsias	475	
Poxviruses	230 × 320	
Influenza virus	85	
T2 *E. coli* bacteriophage	65 × 95	
Tobacco mosaic virus	15 × 300	
Poliomyelitis virus	27	

Source: Prescott, Lansing, John Harley, and Donald Klein. *Microbiology.* 4th ed. McGraw-Hill, 1999.

Bacteria

- Single cell organisms
- Gram-positive and gram-negative
- Ubiquitous in the environment
- Microbiome
- Very rapid growth rates
- Exotoxins and endotoxins

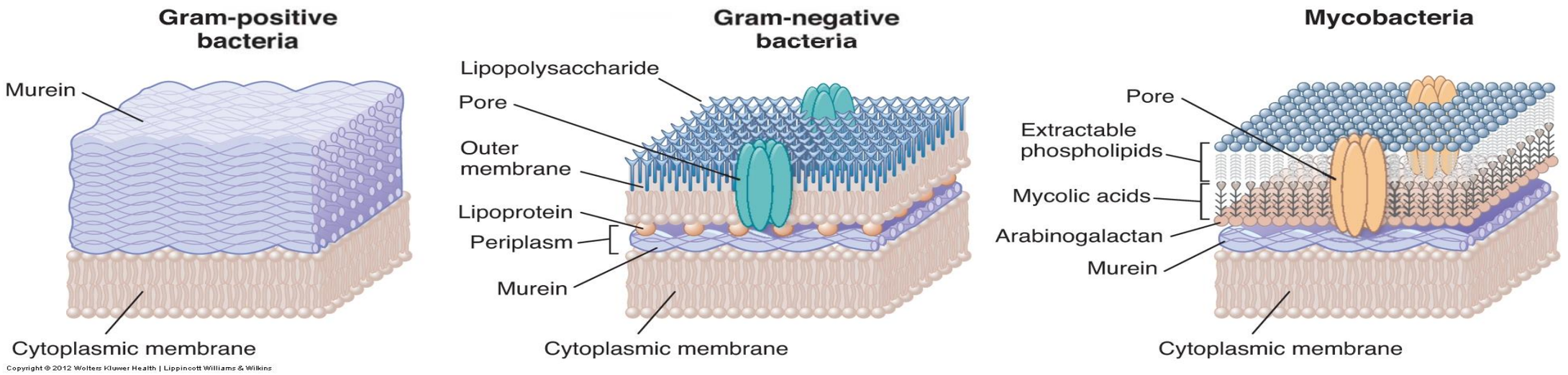
Gram-Staining



Cell Wall

- Provides shape
- Protects against osmotic lysis
- Physical barrier
- Peptidoglycan (Murein)

- NAM-NAG-amino polymer



Source: Golan, David E., Armen H. Tashjian, and Ehrin J. Armstrong, eds. *Principles of Pharmacology: The Pathophysiologic Basis of Drug Therapy*. Lippincott Williams & Wilkins, 2011.

Golan, Fig. 34-1

Cell Wall

Gram-positive vs. gram negative

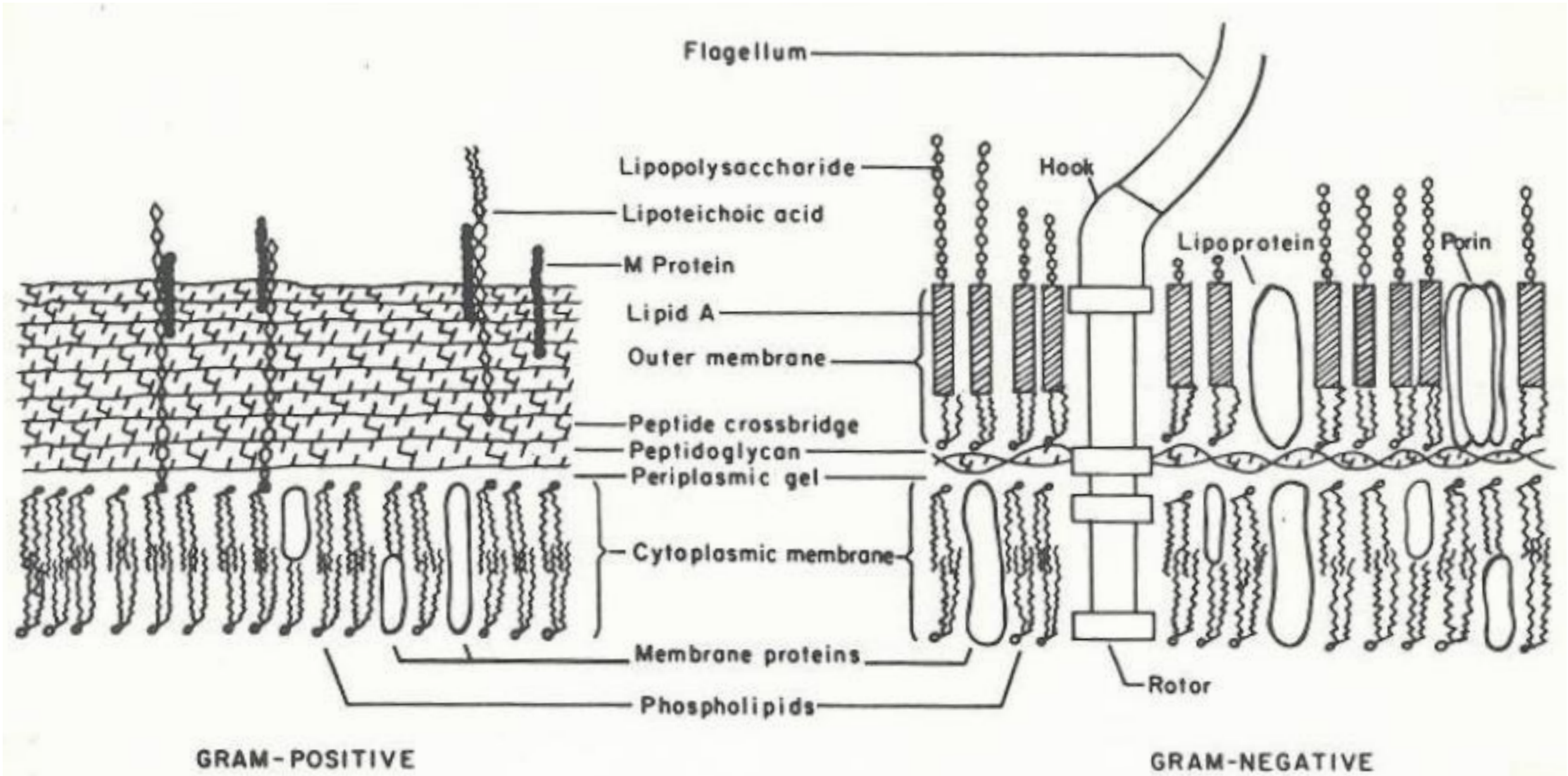


Fig. 1-2. Composition of the cell surfaces of gram-positive and gram-negative bacteria.

Source: White, David, James T. Drummond, and Clay Fuqua. *The Physiology and Biochemistry of Prokaryotes.* 3rd ed. Oxford University Press, 2007.

The Physiology and Biochemistry of Prokaryotes, 3rd Ed.,White

Cell Wall

Gram-positive vs. gram negative

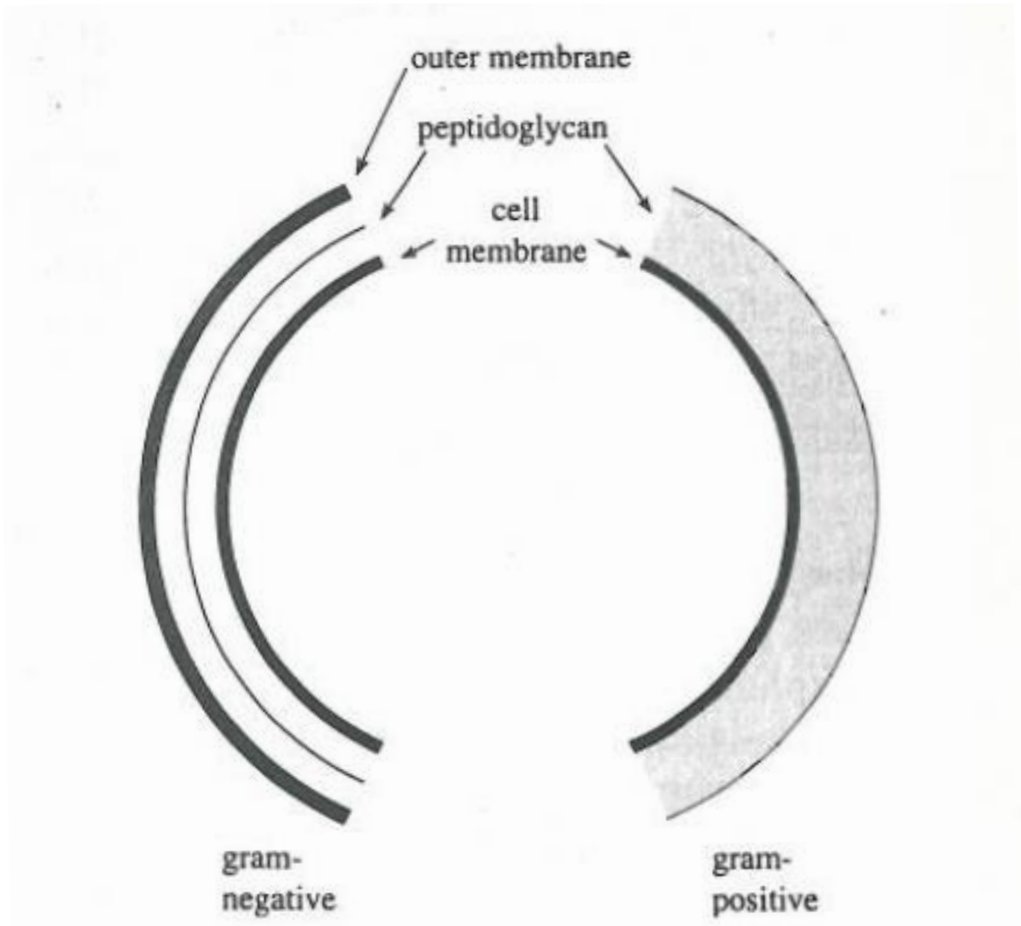
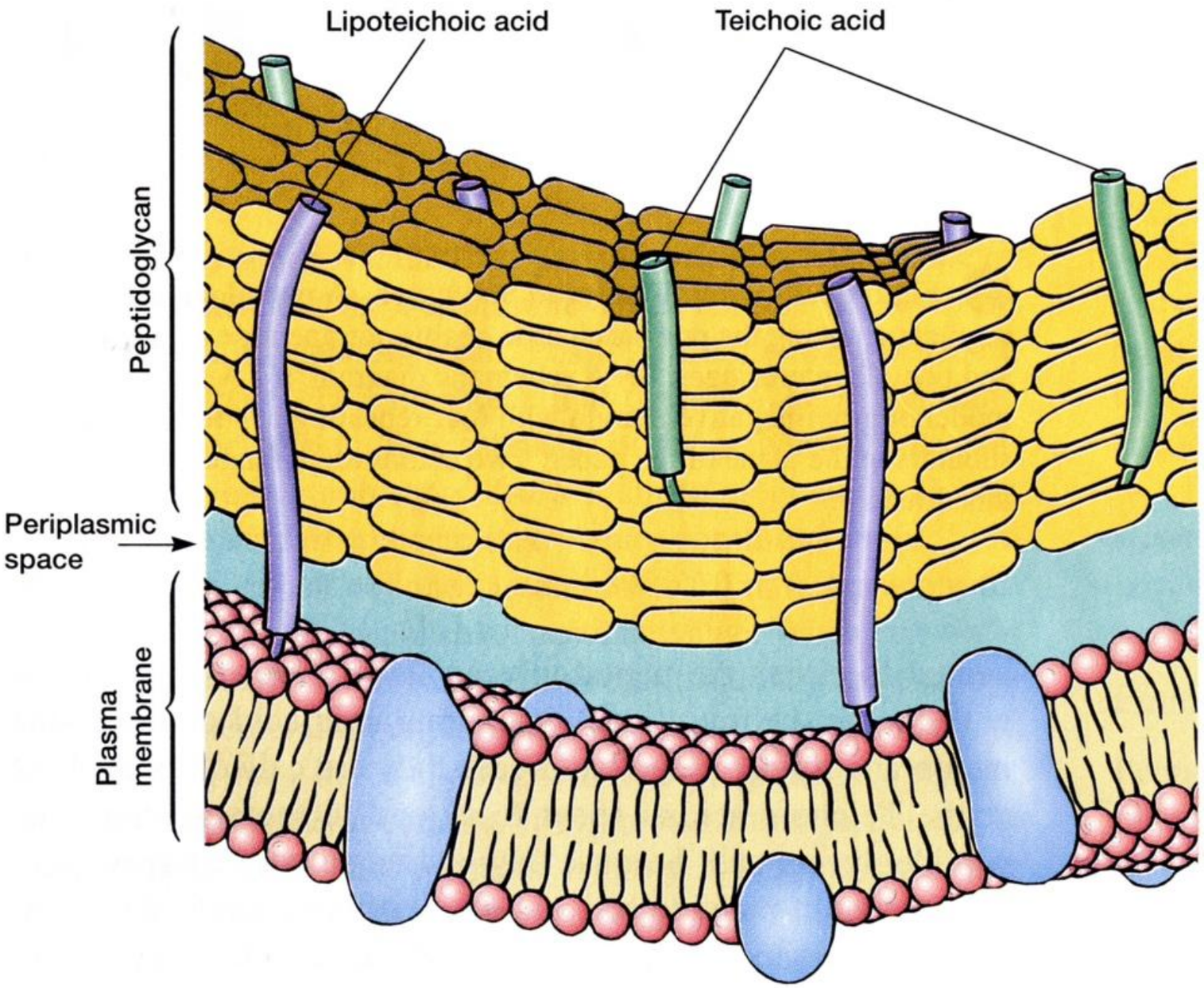


Fig. 1.7 Schematic illustration of a gram-negative and a gram-positive bacterial cell wall. Note the presence of an outer membrane (also called outer envelope) in the gram-negative wall and the much thicker peptidoglycan layer in the gram-positive wall.

Source: White, David, James T. Drummond, and Clay Fuqua. *The Physiology and Biochemistry of Prokaryotes.* 3rd ed. Oxford University Press, 2007.

The Physiology and Biochemistry of Prokaryotes, 3rd Ed.,White

Gram Positive

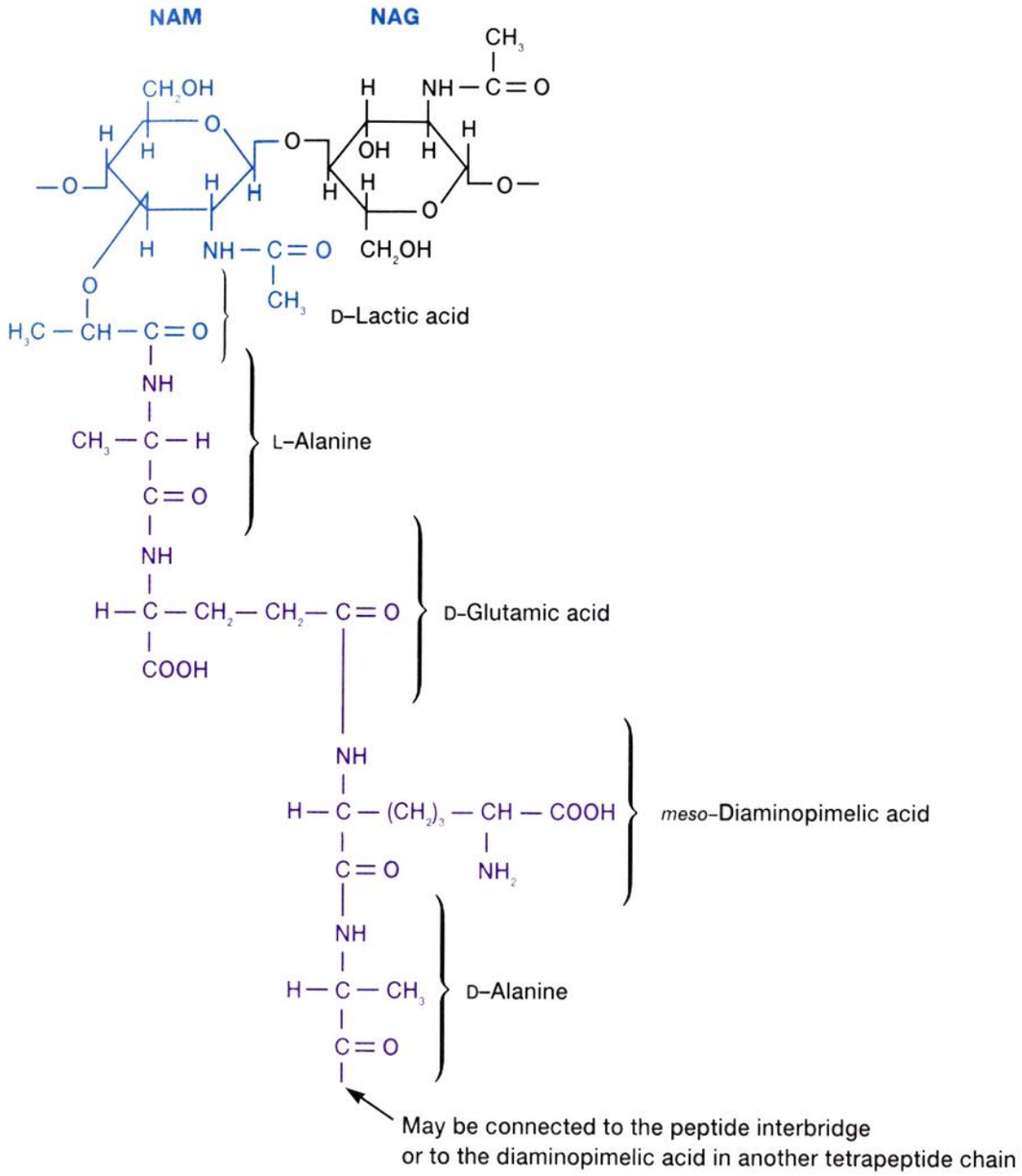


Source: Prescott, Lansing, John Harley, and Donald Klein. *Microbiology*. 4th ed. McGraw-Hill, 1999.

- Gram positive bacteria
- Thick peptidoglycan
- Teichoic acids
  - Contain phosphates
  - Impart negative charge

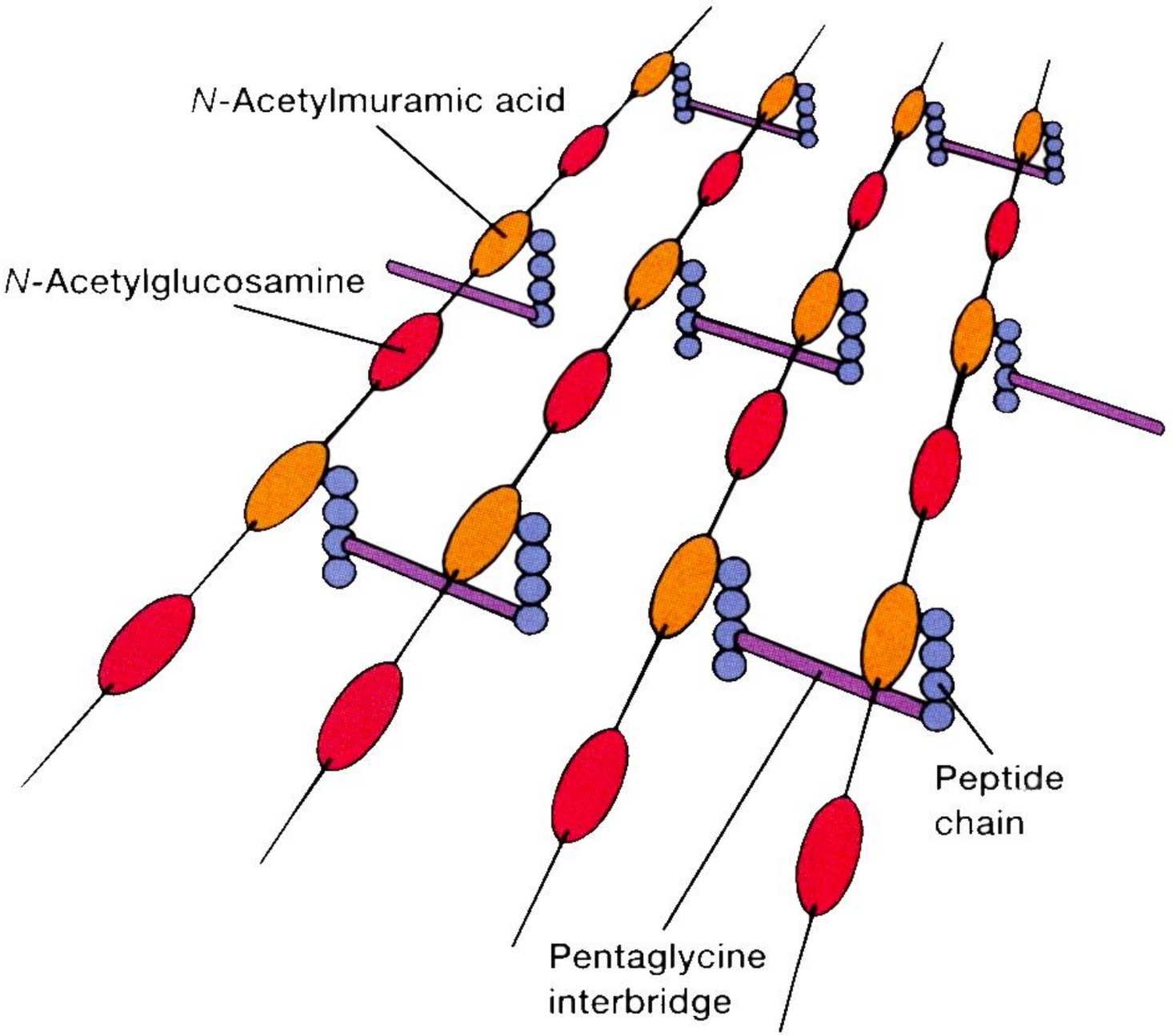
Microbiology, 4th Ed., Prescott

NAM-NAG-Peptide



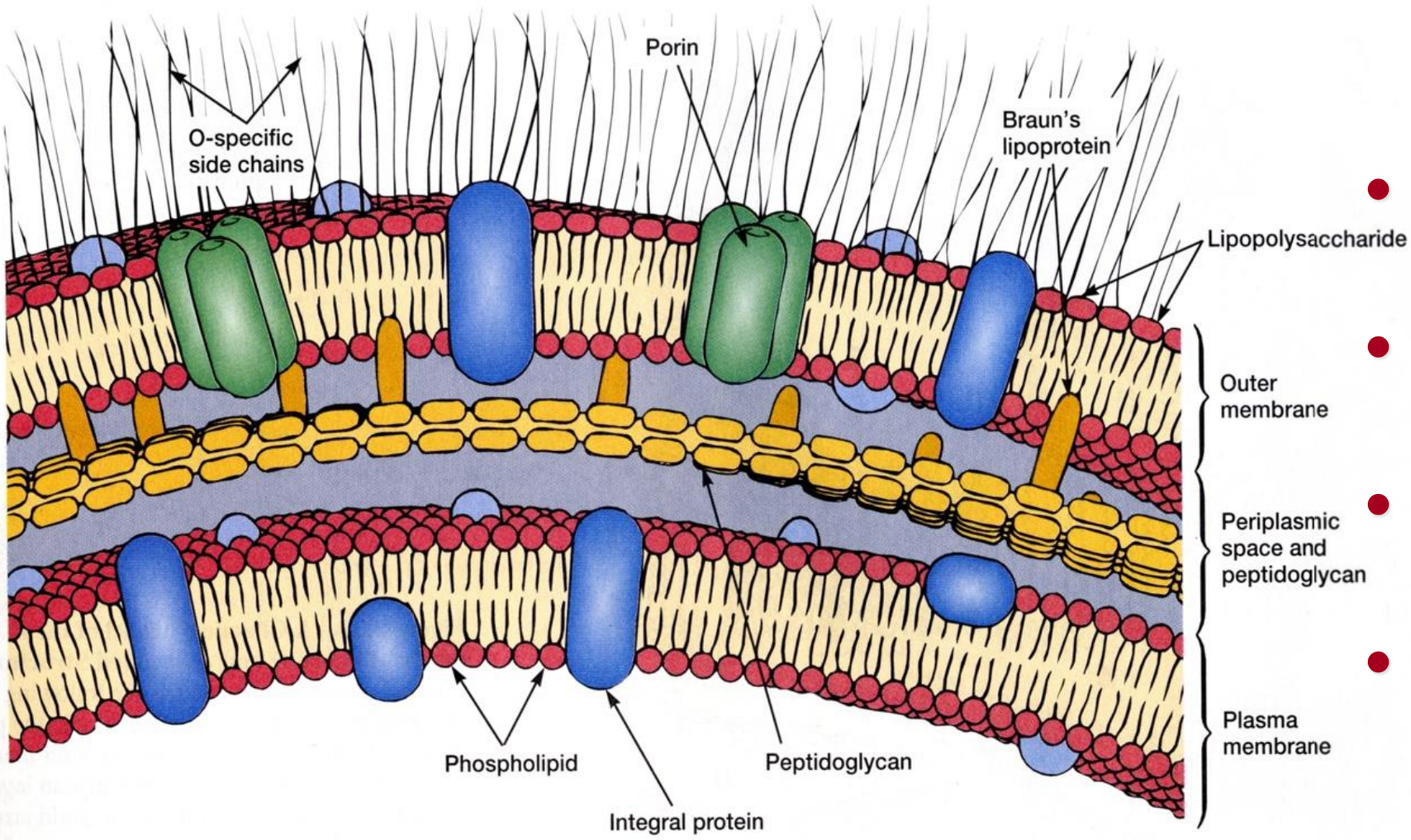
Source: Prescott, Lansing, John Harley, and Donald Klein. *Microbiology*. 4th ed. McGraw-Hill, 1999.

Peptidoglycan



Source: Prescott, Lansing, John Harley, and Donald Klein. *Microbiology*. 4th ed. McGraw-Hill, 1999.

Gram Negative



Source: Prescott, Lansing, John Harley, and Donald Klein. *Microbiology.* 4th ed. McGraw-Hill, 1999.

- **Lipopolysaccharide**
- Highly diverse and changing polysaccharides
  - Avoids host detection
- Limits host interaction with outer membrane
- Prevents entry of bile salts, antibiotics, and toxicants
- Prevents loss of nutrients from periplasmic space
- **Transporters and porins**
  - Selectively export and uptake small molecules

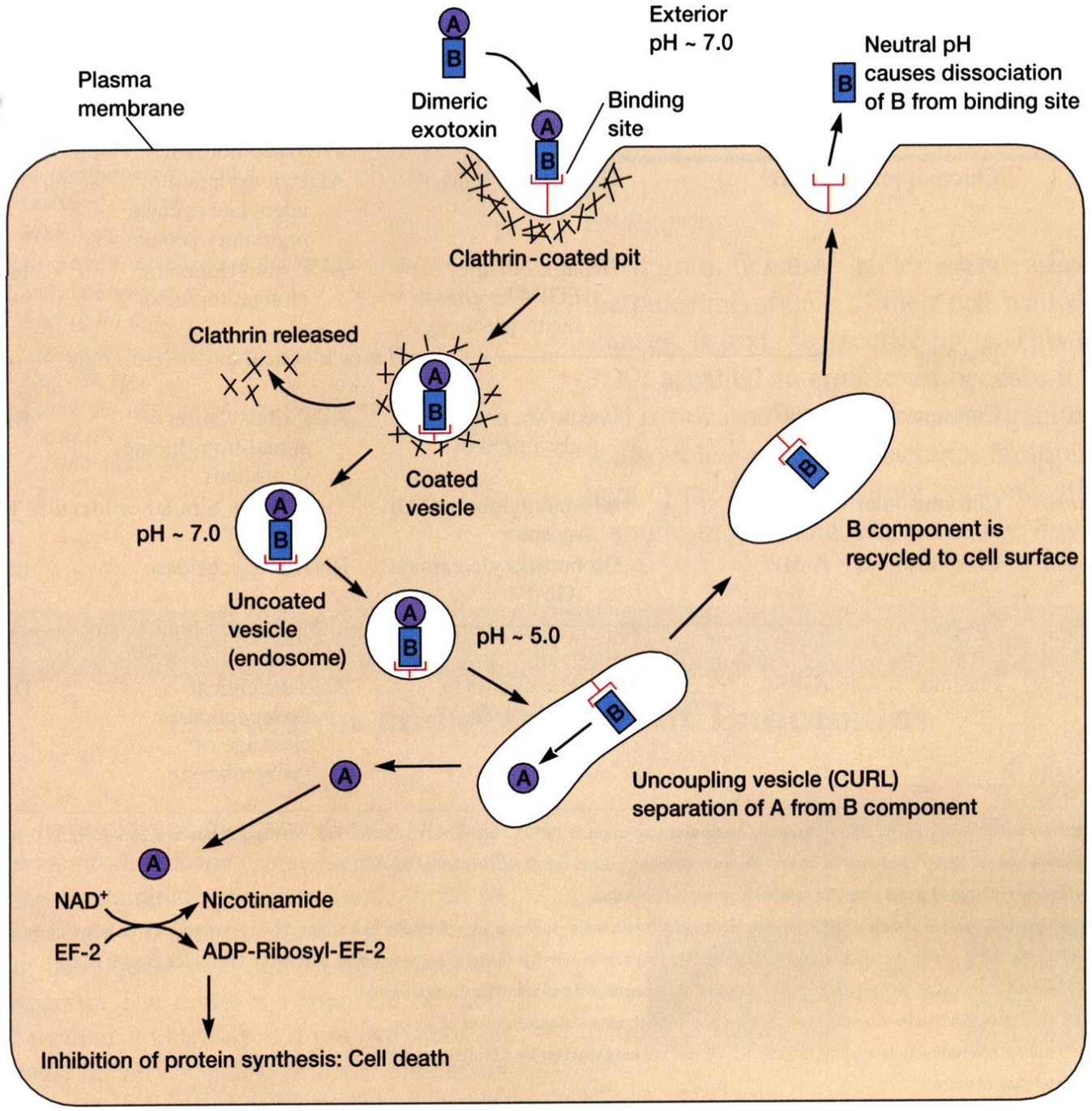
Microbiology, 4th Ed., Prescott

Exotoxins and Endotoxins

- Exotoxins
  - Heat-labile, proteins released into surroundings
  - Can migrate to different cells or tissues
    - Diphtheria toxin, anthrax toxin, cholera toxin
- Endotoxins
  - Heat-stable lipopolysaccharide
  - Outer membrane of gram-negative bacteria
  - Released during lysis or cell division/growth
  - Leads to blood clotting, hemorrhaging and organ failure

Exotoxin: Diphtheria Toxin

- *Corynebacterium diphtheriae*
  - Gram-positive, facultative anaerobe
- Diphtheria toxin:
- 62 kDa Protein
- B: Cell surface receptor binding
- A: Enzymatic region
- Catalyzes addition of ADP-Ribose to EF2
- Inhibits translation



Source: Prescott, Lansing, John Harley, and Donald Klein. *Microbiology*. 4th ed. McGraw-Hill, 1999.

Antibiotic resistance is and will be a problem

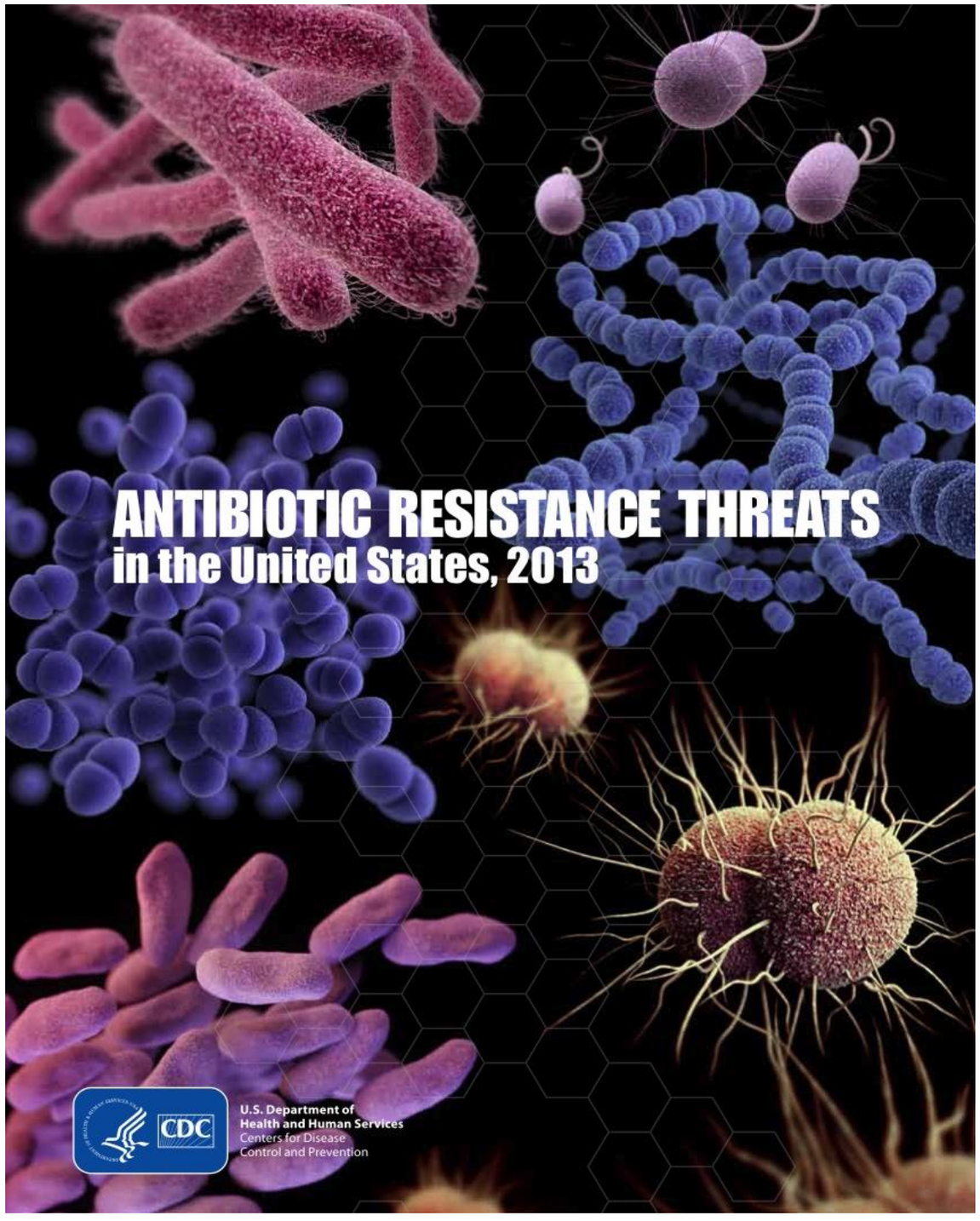
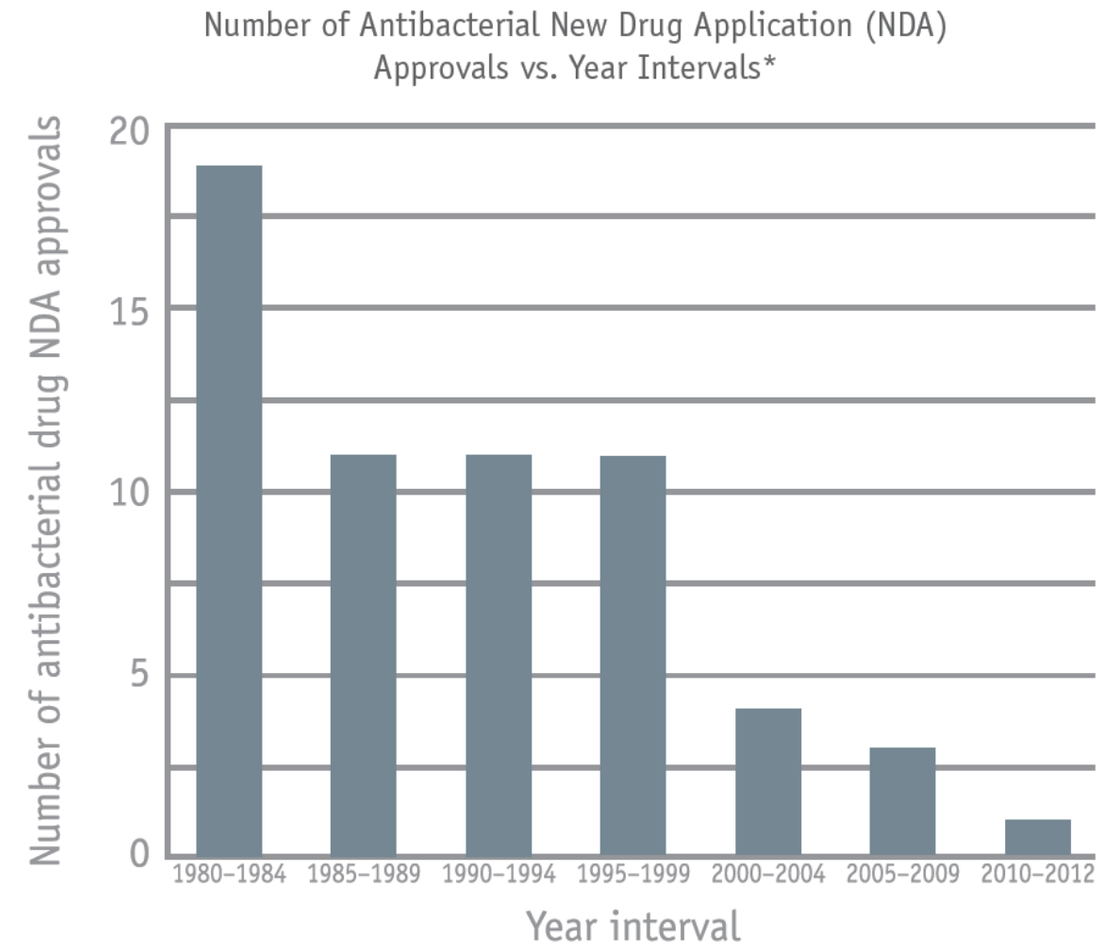


Image is by the Centers for Disease Control and Prevention, and is in the public domain.

Antibiotic Drug Pipeline

Tomorrow's Antibiotics: The Drug Pipeline

The number of new antibiotics developed and approved has steadily decreased in the past three decades, leaving fewer options to treat resistant bacteria.



*Intervals from 1980–2009 are 5-year intervals; 2010–2012 is a 3-year interval. Drugs are limited to systemic agents.
Data courtesy of FDA' s Center for Drug Evaluation and Research (CDER).

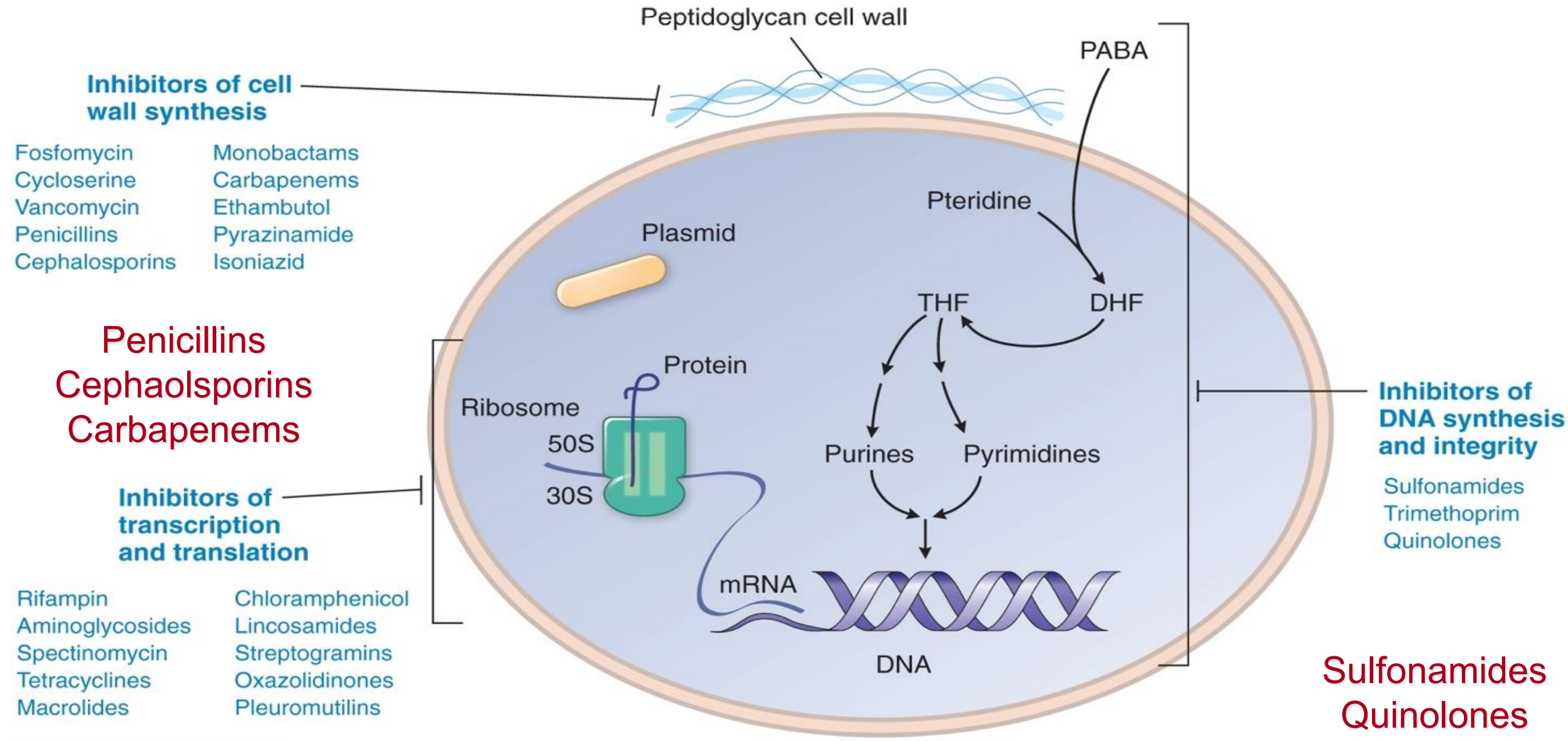
Image is by the Centers for Disease Control and Prevention, and is in the public domain.

Antibiotic Resistance Threats in the United States, 2013, CDC

How to Target Bacteria?

- Unique processes/proteins
- Cell Wall
- DNA synthesis
  - Single circular dsDNA chromosome
- Ribosomes
- Can you selectively target pathogenic bacteria?

How to Target Bacteria?



Penicillins
Cephaolsporins
Carbapenems

Sulfonamides
Quinolones
Fluroquiolones

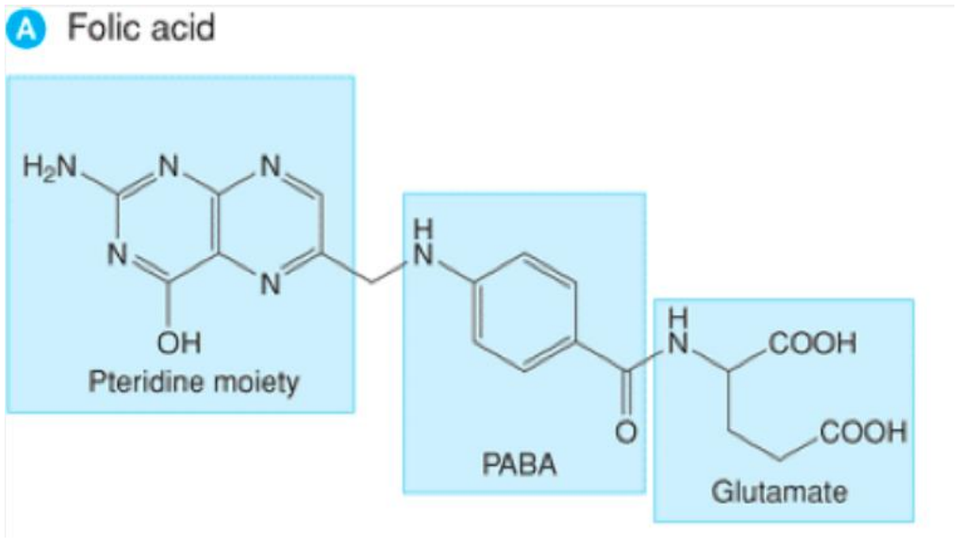
Aminoglycosides
Macrolides

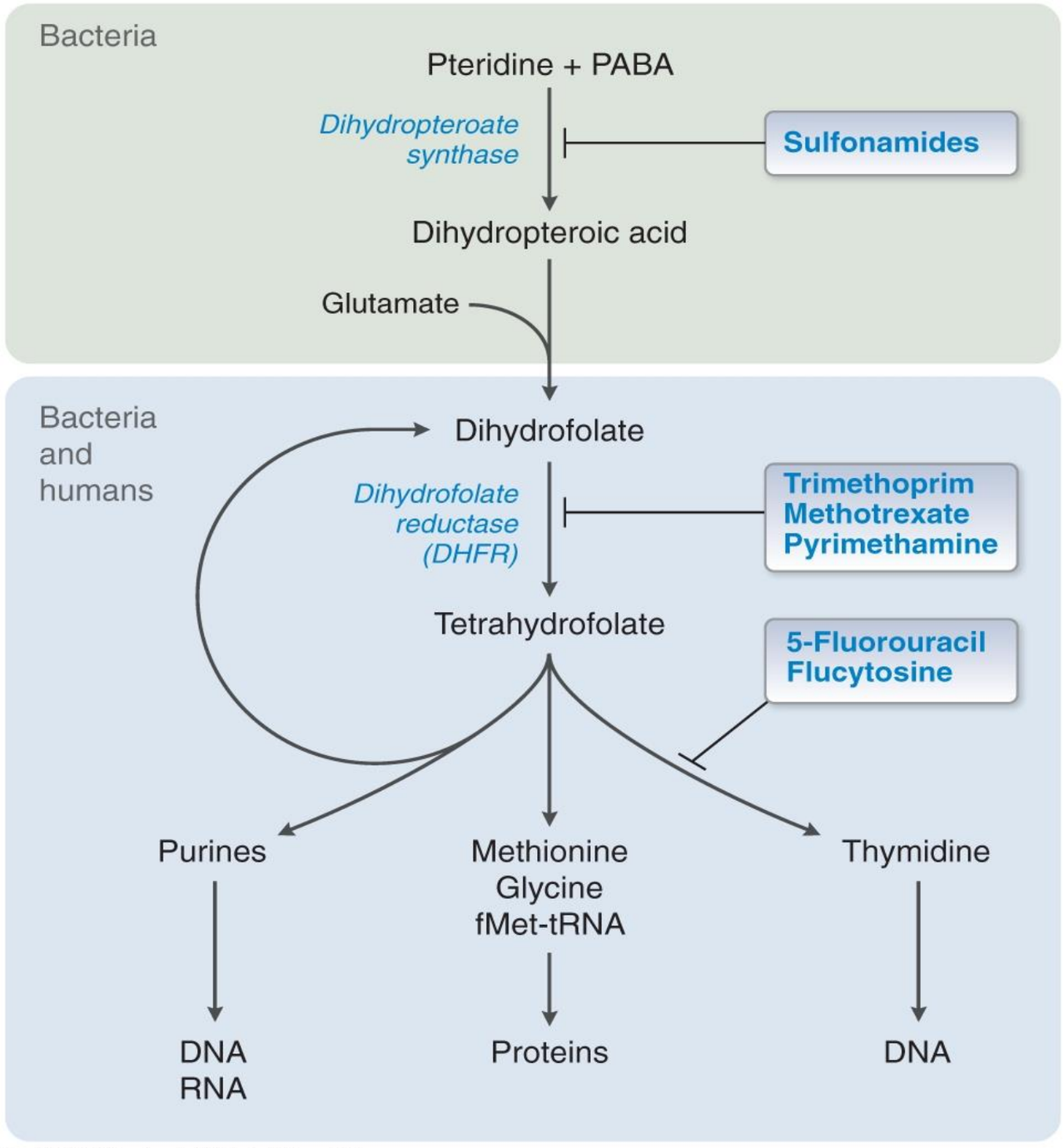
Reactive Oxygen Species

Golan, Fig. 32-1

Folic Acid Metabolism

- Humans require folic acid in diet and use as a cofactor in the synthesis of amino acids and nucleic acids
- Bacteria make their own folic acid
- Bacteriostatic





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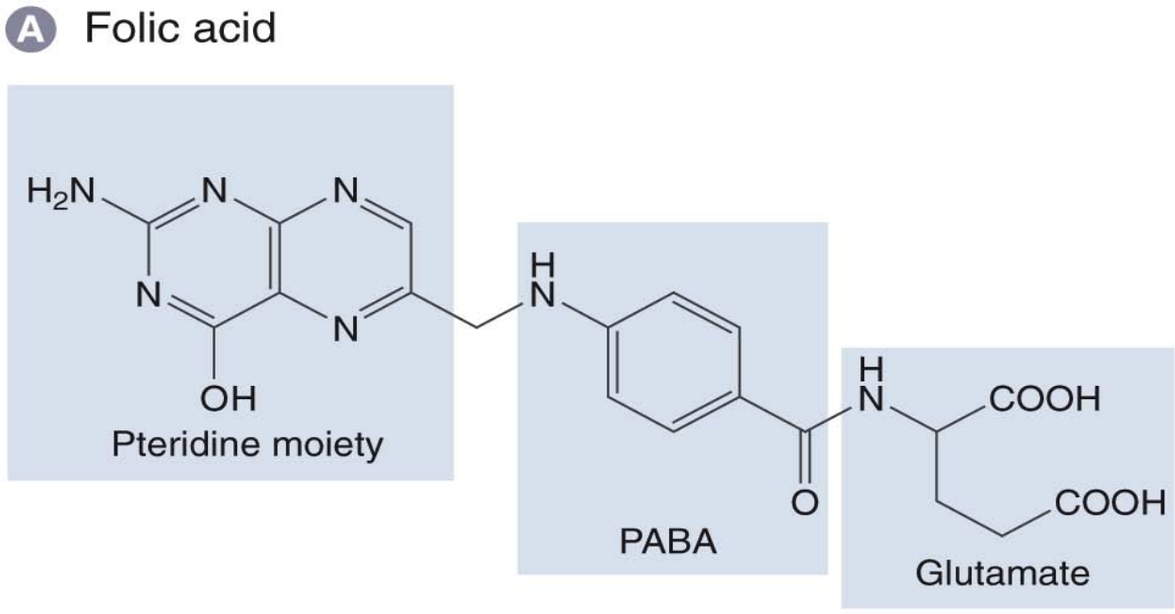
Source: Golan, David E., Armen H. Tashjian, and Ehrin J. Armstrong, eds. *Principles of Pharmacology: The Pathophysiologic Basis of Drug Therapy*. Lippincott Williams & Wilkins, 2011.

Golan, Fig. 32-6,32-7

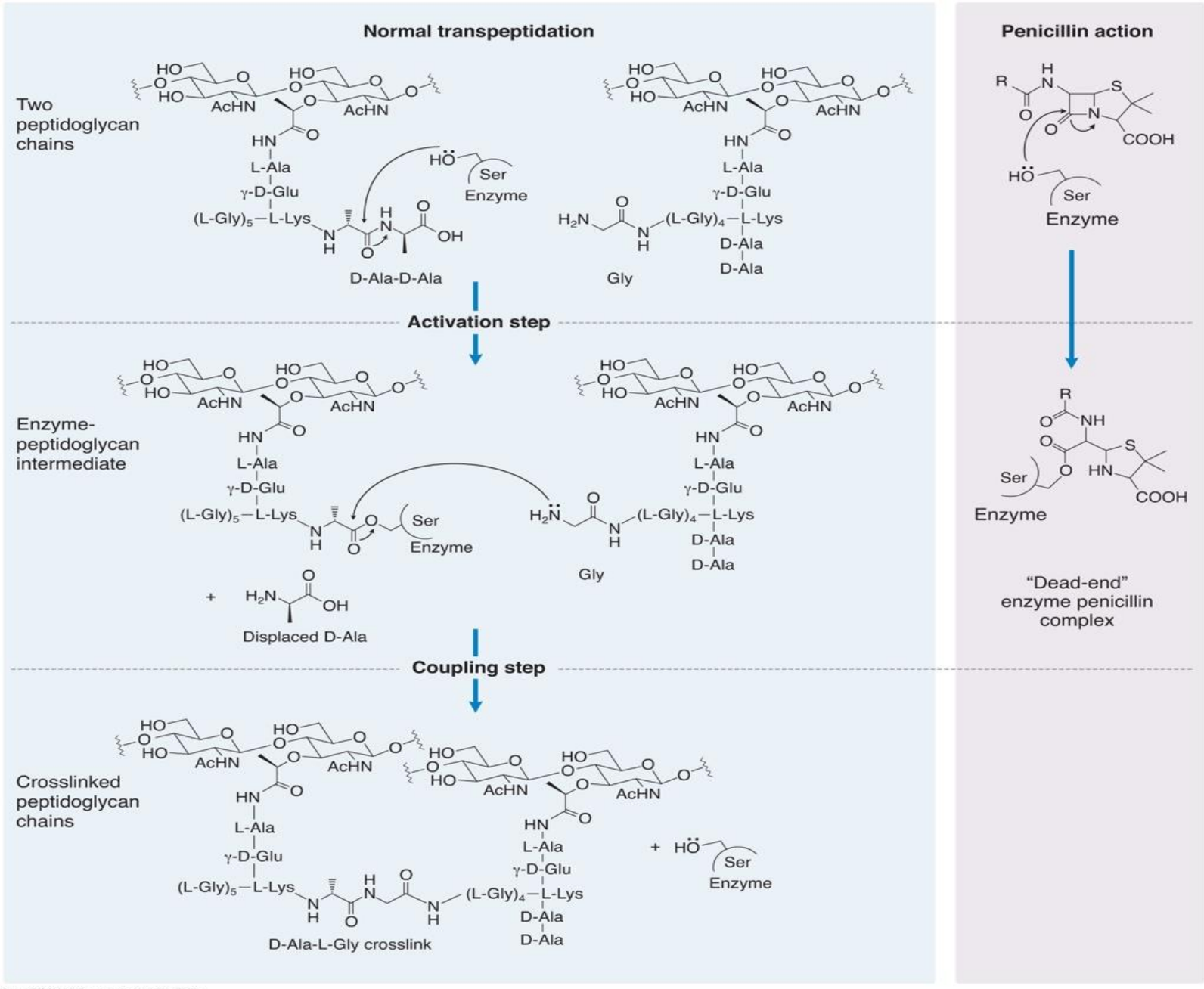
Sulfonamides



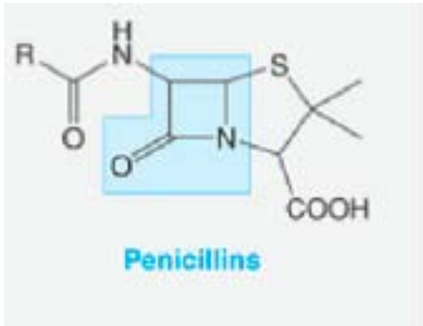
Source: Golan, David E., Armen H. Tashjian, and Ehrin J. Armstrong, eds. *Principles of Pharmacology: The Pathophysiologic Basis of Drug Therapy*. Lippincott Williams & Wilkins, 2011.

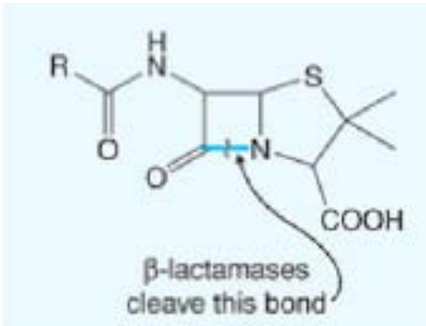
Golan, Fig. 32-6

β-Lactams



- Inhibit cell wall polymer crosslinking
- Inhibit transpeptidase
- Bactericidal

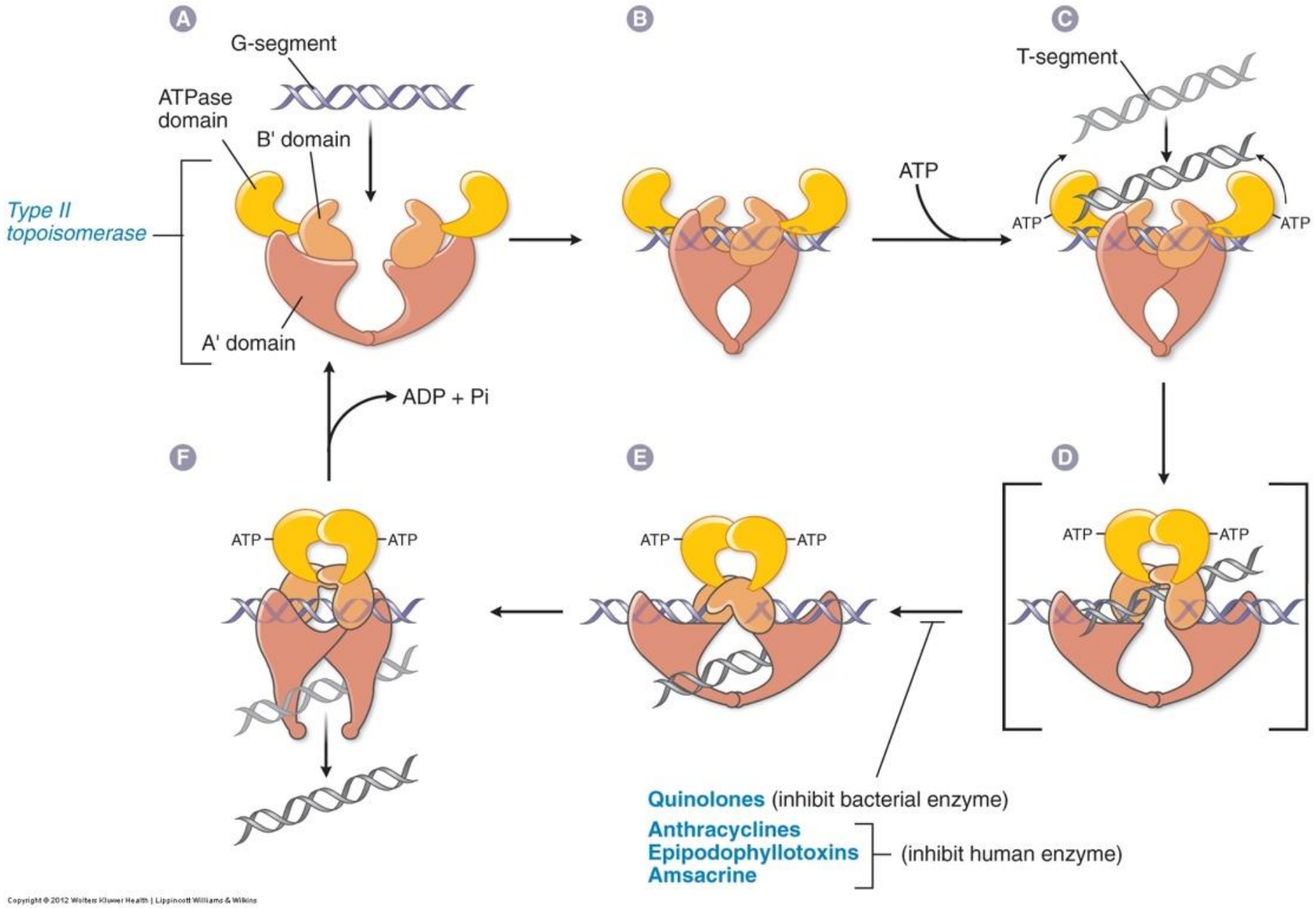




Source: Golan, David E., Armen H. Tashjian, and Ehrin J. Armstrong, eds. *Principles of Pharmacology: The Pathophysiologic Basis of Drug Therapy*. Lippincott Williams & Wilkins, 2011.

Golan, Fig. 34-3, 34-6

Quinolones/Fluoroquinolones



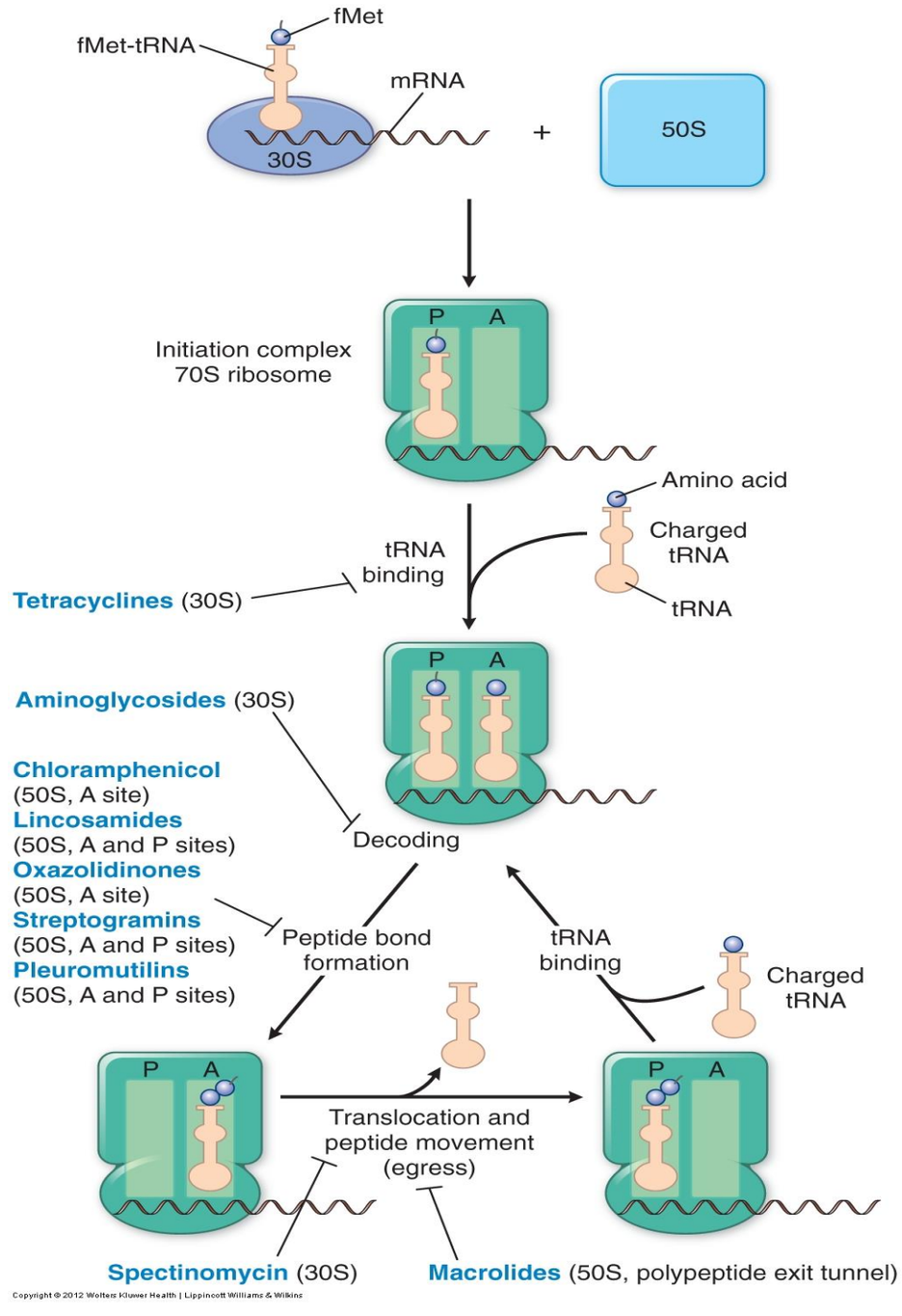
- **Type II Topoisomerase**
- Produce double-strand breaks in DNA
- Quinolones inhibit TopoII before second strand can pass
- Bactericidal

Source: Golan, David E., Armen H. Tashjian, and Ehrin J. Armstrong, eds. *Principles of Pharmacology: The Pathophysiologic Basis of Drug Therapy*. Lippincott Williams & Wilkins, 2011.

Golan, Fig. 33-4

Inhibiting Protein Synthesis

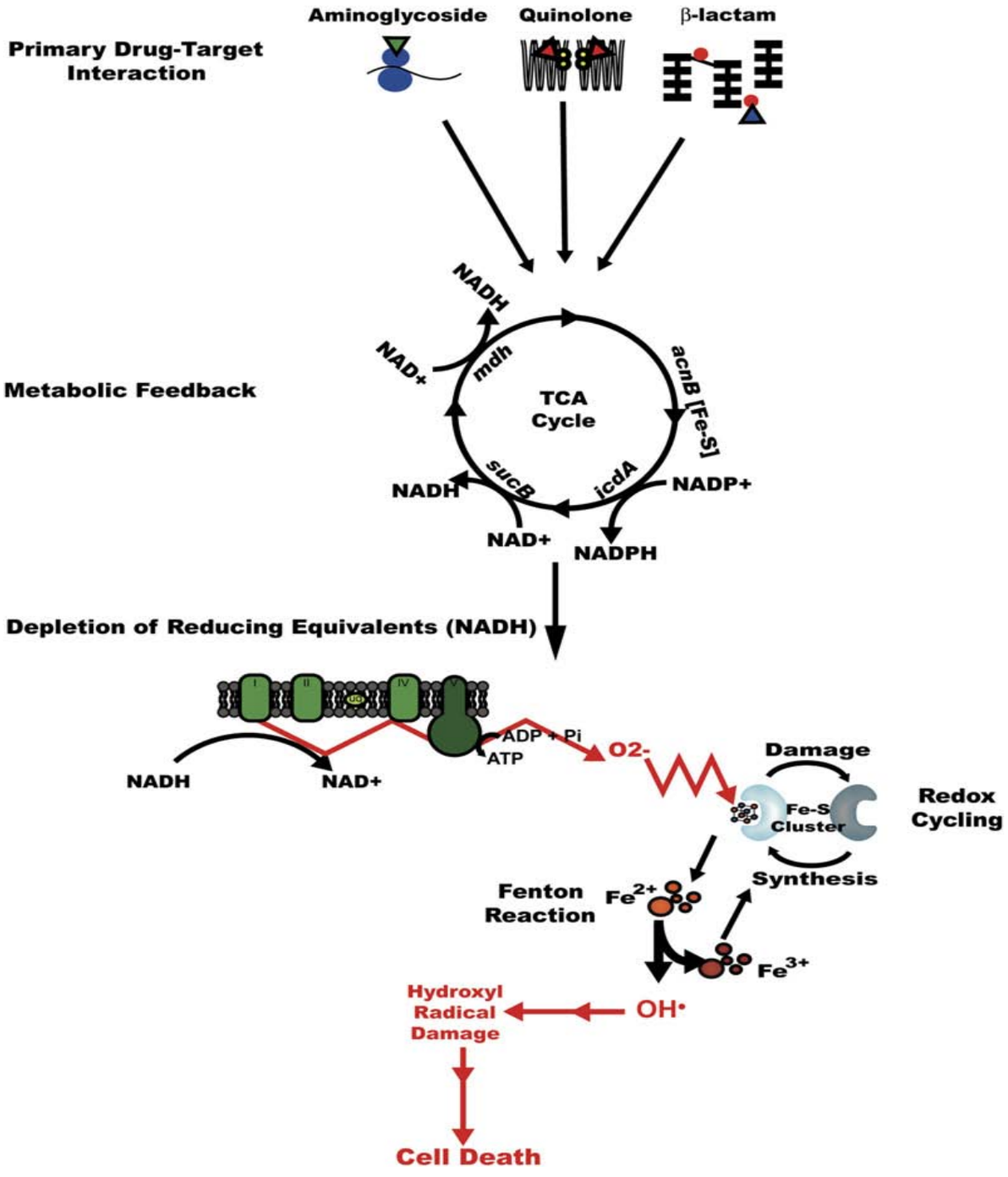
- Multiple Mechanisms
- Not completely understood
- Tetracyclines, Macrolides, Chloramphenicol, Oxazolidinones are bacteriostatic
- Aminoglycosides are only bactericidal class among the protein synthesis inhibitors



Source: Golan, David E., Armen H. Tashjian, and Ehrin J. Armstrong, eds. *Principles of Pharmacology: The Pathophysiologic Basis of Drug Therapy*. Lippincott Williams & Wilkins, 2011.

Golan, Fig. 33-7

Reactive Oxygen Species

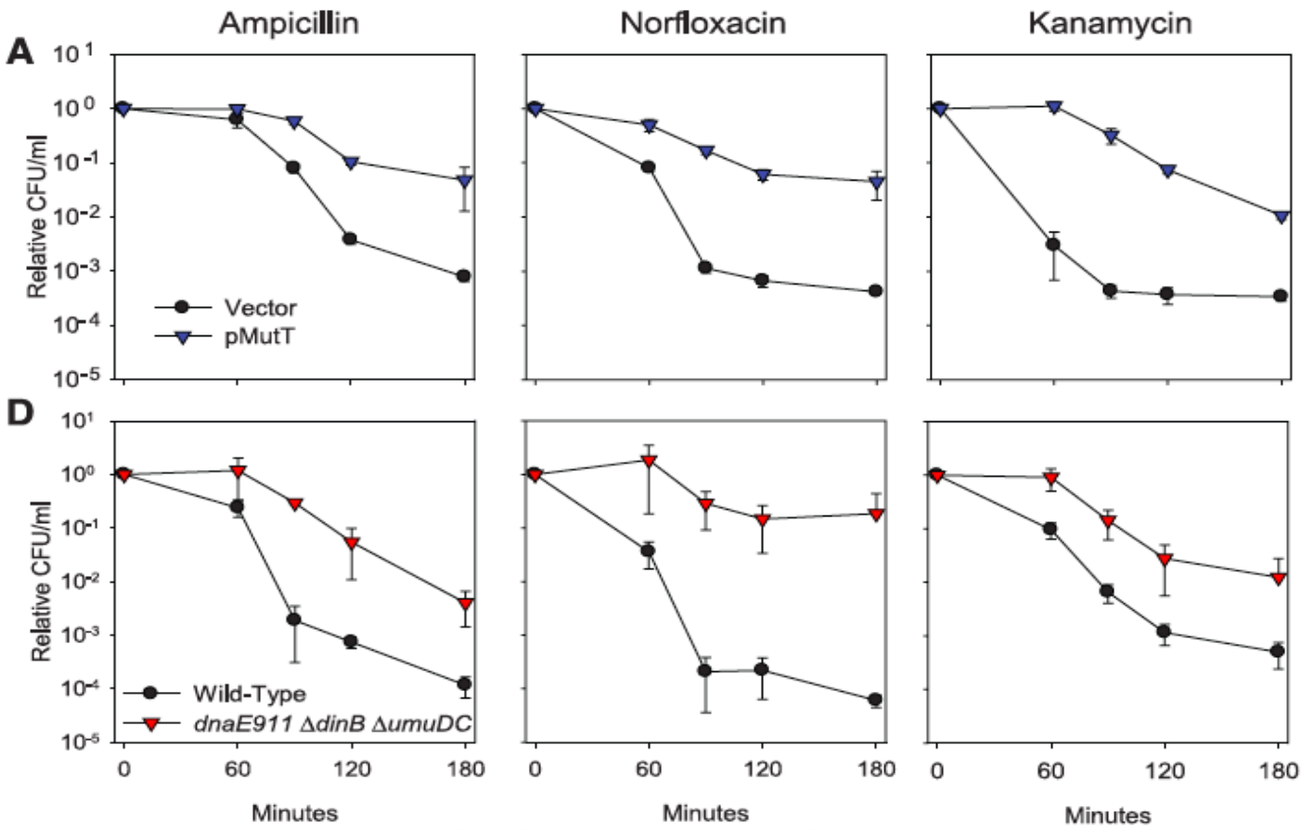


- Observed for bactericidal but not bacteriostatic antibiotics
- NADH depletion
- Dependent on TCA Cycle
- Increased production of superoxide ($O_2^{-\bullet}$)
- Damage to iron-sulfur clusters
- Fenton Chemistry

Source: Kohanski, Michael A., Daniel J. Dwyer, et al. "A Common Mechanism of Cellular Death Induced by Bactericidal Antibiotics." *Cell* 130, no. 5 (2007): 797-810.

Cell, 2007

Reactive Oxygen Species



Source: Foti, James J., Babho Devadoss, et al. "Oxidation of the Guanine Nucleotide Pool Underlies Cell Death by Bactericidal Antibiotics." *Science* 336, no. 6079 (2012): 315-9.

- Increase in ROS leads to an increase in nucleotide pool damage products ultimately producing DNA damage and cell death
- MutT removes 8-oxo-dGTP from the nucleotide pool
- dnaE911, dinB, & umuDC are DNA polymerases that incorporate 8-oxo-dG

8-oxo-dGTP

↓ Mut T

8-oxo-dGMP

Science, 2012, 336, 315-319

Antibiotics

- Unique processes/proteins
- Cell Wall
- DNA synthesis
- Ribosomes
- ROS
- Innate immunity
  - Phagocytes (neutrophils and macrophages)
- Adaptive immunity

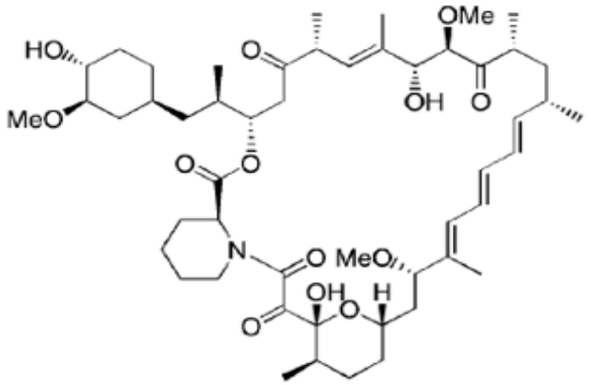
Alexander Fleming

- 1928 - Fleming's discovery of "mold juice"
- Staphylococcus cultures contaminated with a mold from the genus *Penicillium*
- Penicillin was born

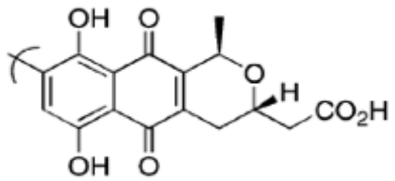
“Mold Juice”

- Why would a mold make a bactericidal compound?
- Why would bacteria make bactericidal compounds?

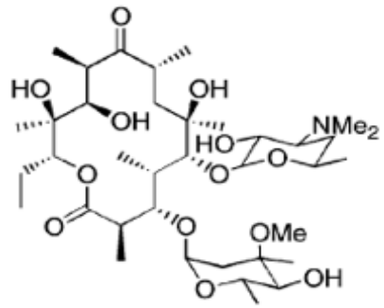
Common Toxins



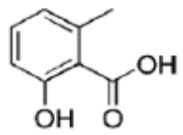
Rapamycin
polyene immunosuppressant



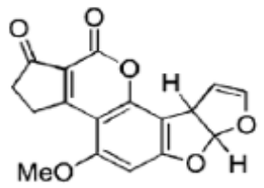
Actinorhodin
aromatic antibiotic



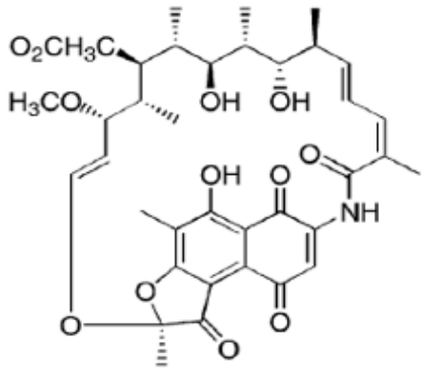
Erythromycin A
macrolide antibiotic



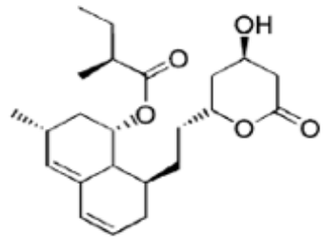
6-Methylsalicylic acid
aromatic antibiotic



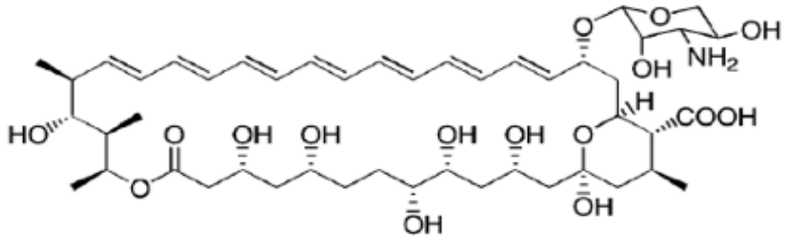
Aflatoxin B1
mycotoxin carcinogen



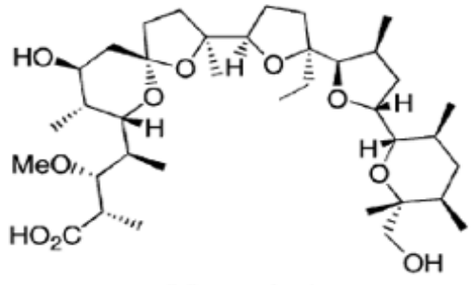
Rifamycin S
ansamycin antibiotic



Lovastatin
anti-cholesterol



Amphotericin B
polyene antifungal



Monensin A
polyether antibiotic

Source: Staunton, James, and Kira J. Weissman. "Polyketide Biosynthesis: A Millennium Review." *Natural Product Reports* 18, no. 4 (2001): 380-416.

Nat. Prod. Rep., 2001, 18, 380

Polyketide Biosynthesis

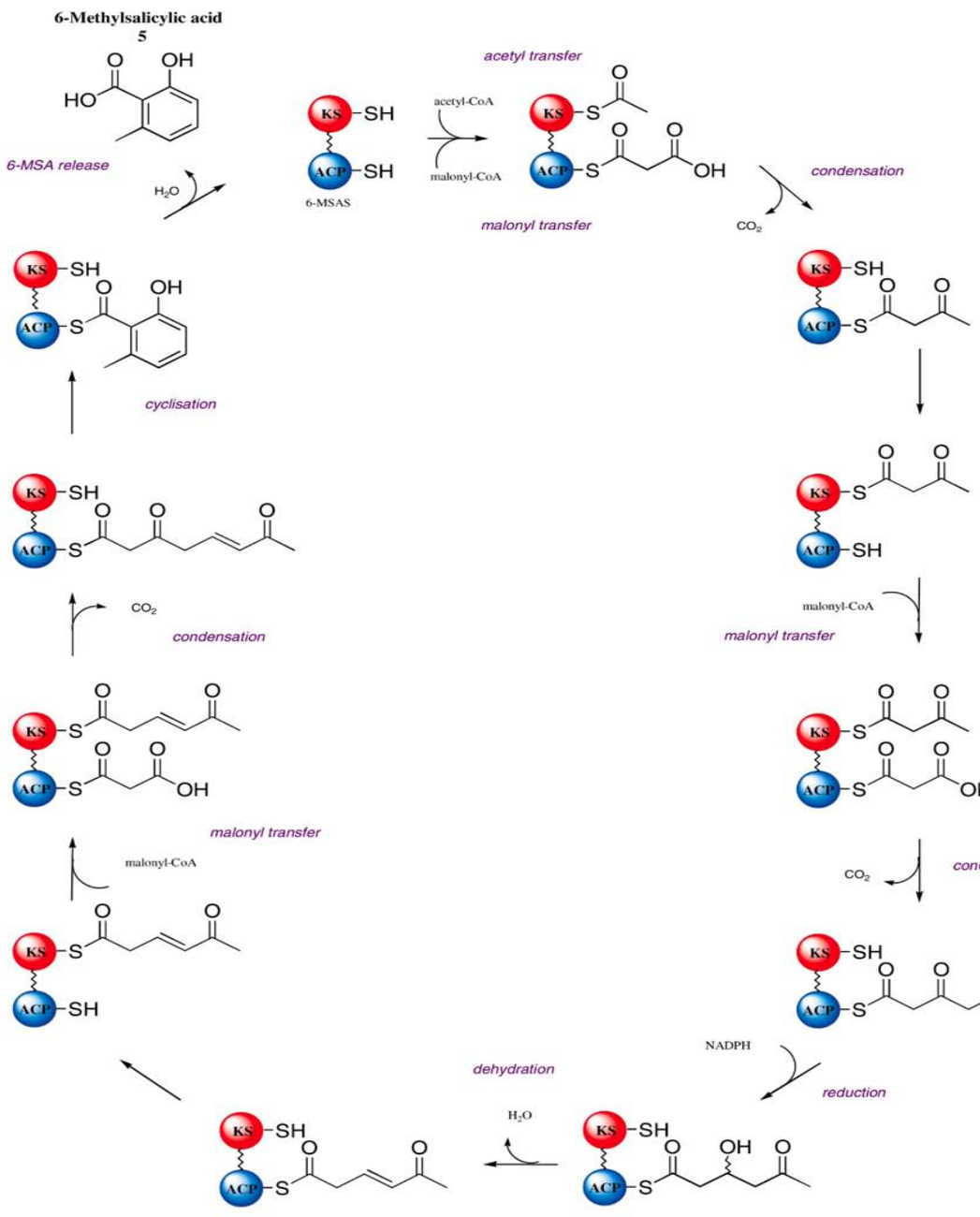
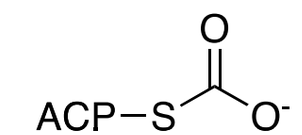
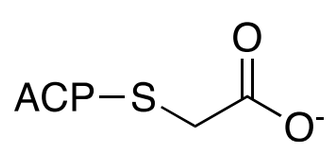


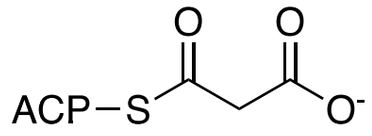
Fig. 23 The biosynthetic pathway for the fungal polyketide 6-methylsalicylic acid (6-MSA) **5**.



- Acetate



- Propionate

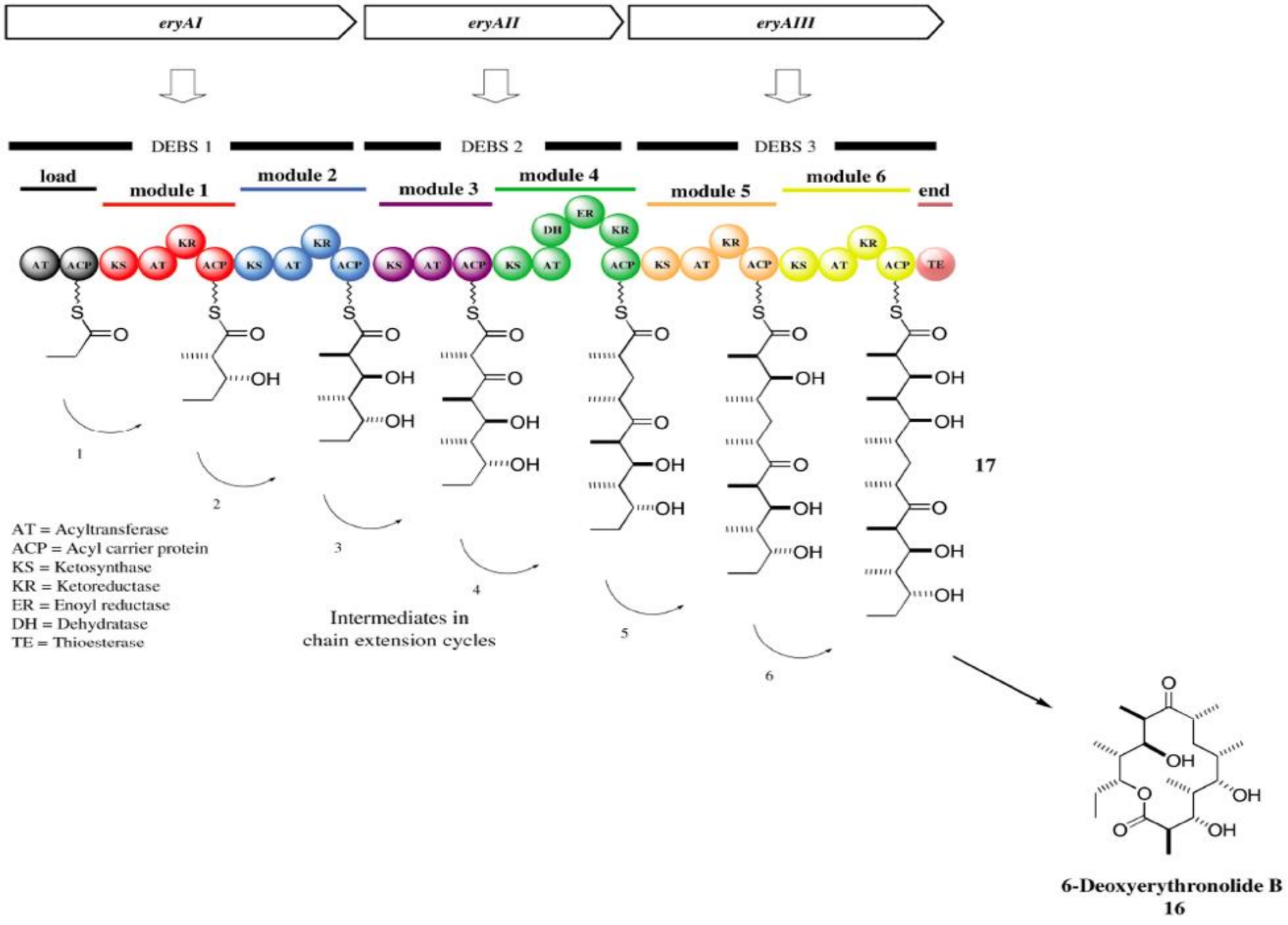


- Malonate

Source: Staunton, James, and Kira J. Weissman. "Polyketide Biosynthesis: A Millennium Review." *Natural product reports* 18, no. 4 (2001): 380-416.

Nat. Prod. Rep., 2001, 18, 380

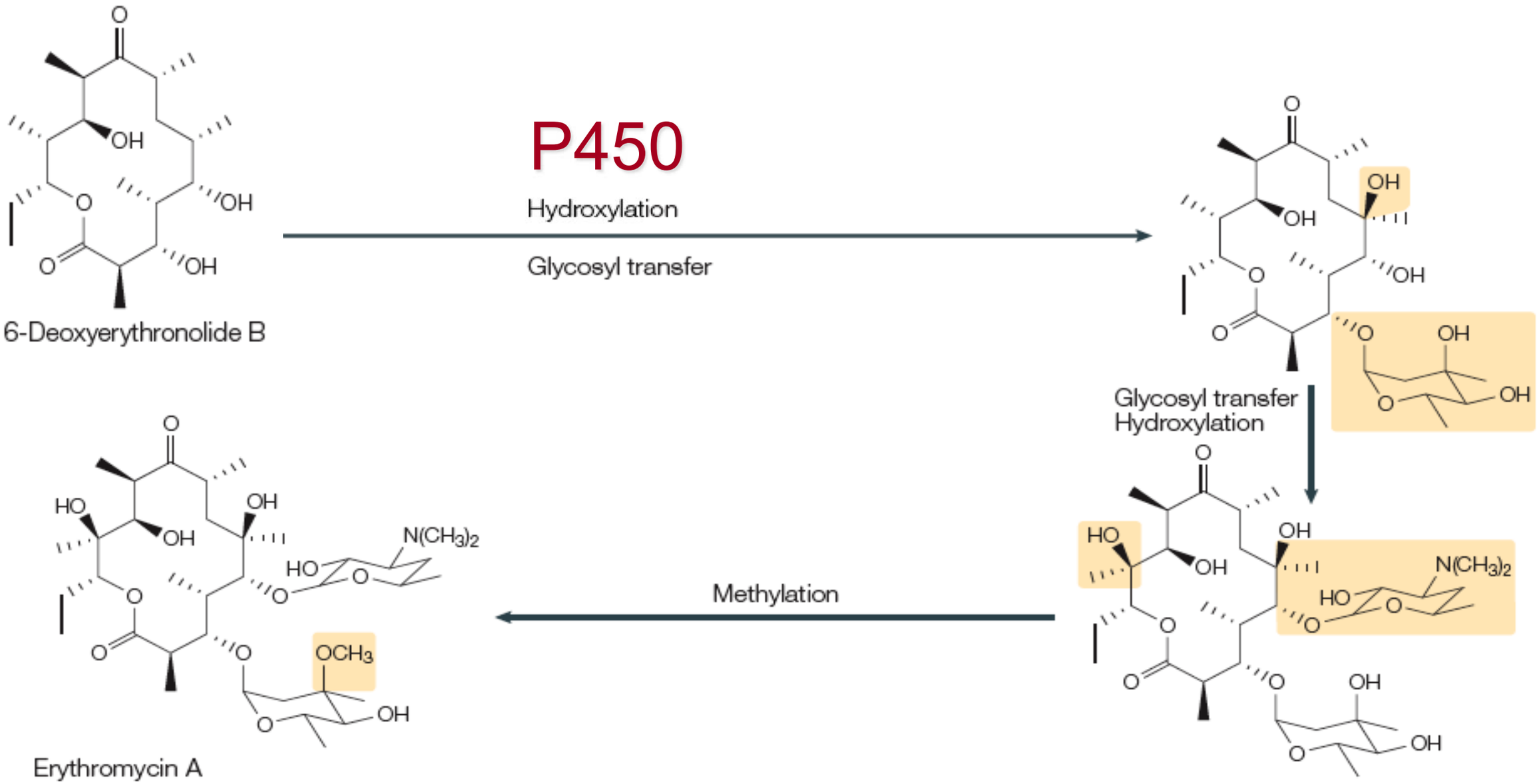
Polyketide Biosynthesis



Source: Staunton, James, and Kira J. Weissman. "Polyketide Biosynthesis: A Millennium Review." *Natural Product Reports* 18, no. 4 (2001): 380-416.

Nat. Prod. Rep., 2001, 18, 380

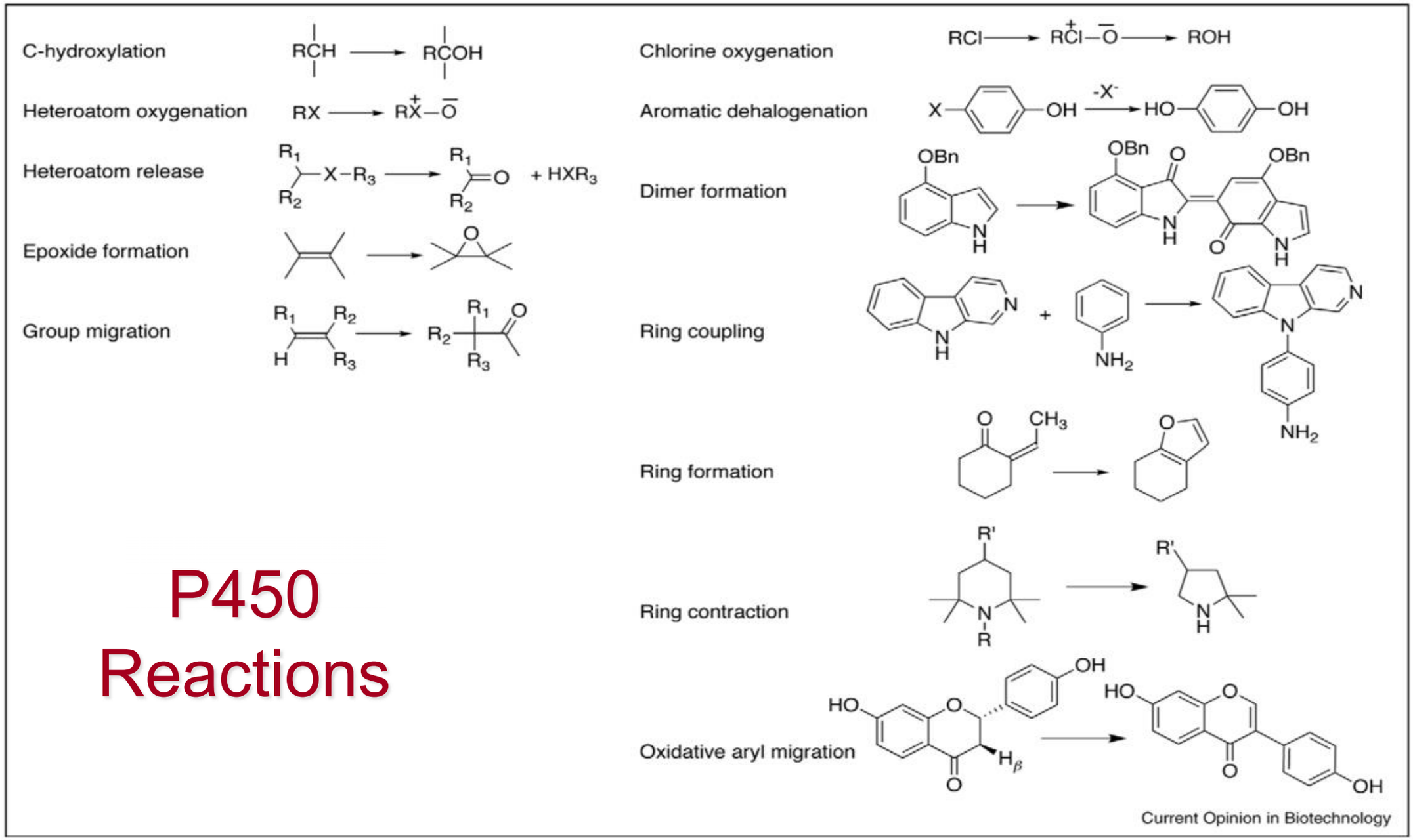
Post-PKB Modifications



Courtesy of Nature Publishing Group. Used with permission.
Source: Weissman, Kira J., and Peter F. Leadlay. "Combinatorial Biosynthesis of Reduced Polyketides."
Nature Reviews Microbiology 3, no. 12 (2005): 925-36.

Nat. Rev., 3, 2005, 925

Post-PKB - *Secondary Metabolites*



P450 Reactions

Source: Lamb, David C., Michael R. Waterman, et al. "Cytochromes P450 and Drug Discovery." *Current opinion in biotechnology* 18, no. 6 (2007): 504-12.

Curr Opin. Biotech., 18, 2007, 504

Bacterial Treatment

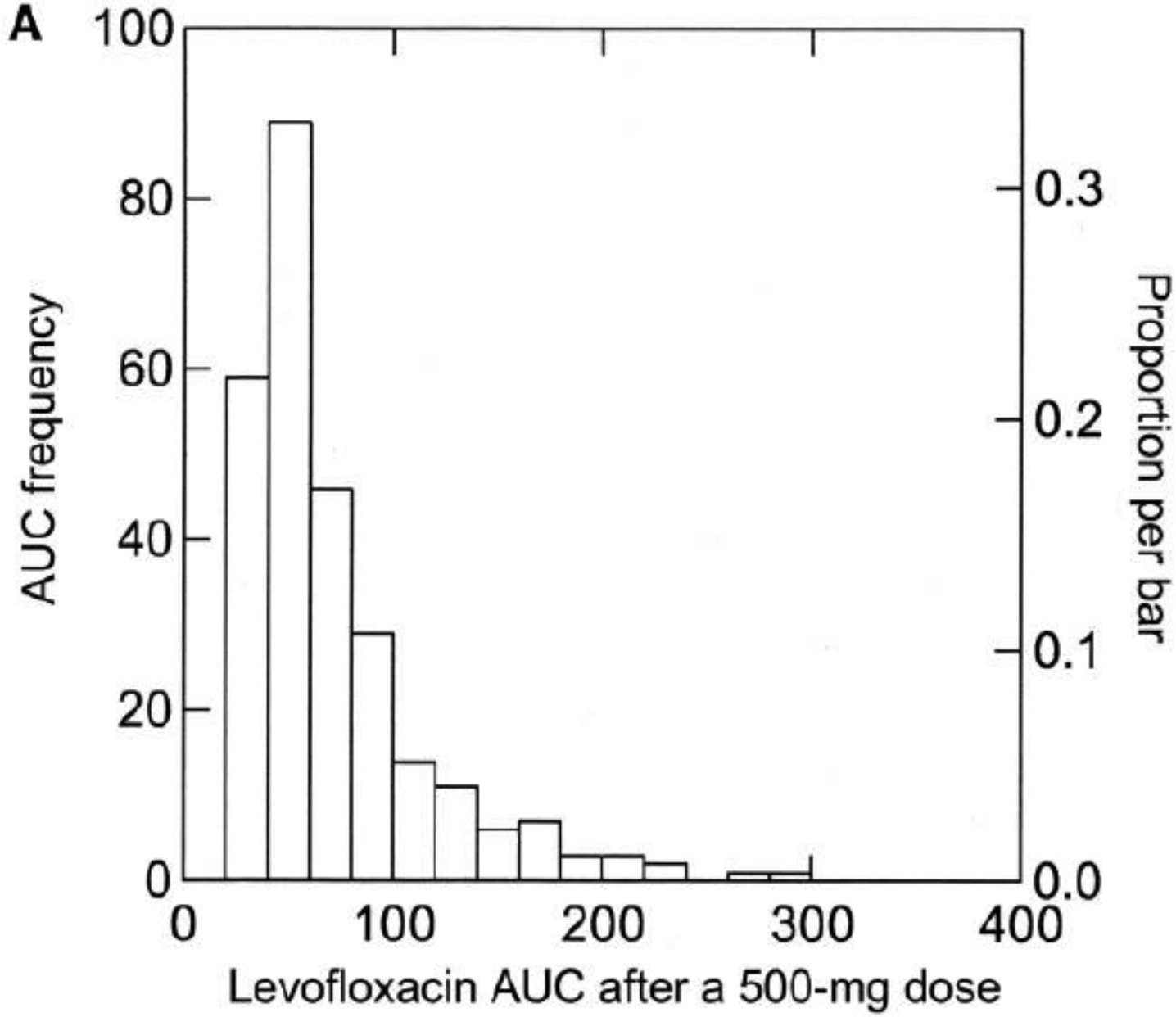
- Two main initiatives for human infection
  - Eliminate bacteria
  - Avoid emergence of resistance
- Bacteriostatic vs. Bactericidal
- During preclinical (and sometimes clinical) development the compound's efficacy can be measured (not the case for many targets)
- Different drug classes require different dosing (i.e., different measurable endpoints)
- MIC = Minimal Inhibitory Concentration

Clinical Bacterial Pharmacology

Acquire data:

- PK (AUC, Cmax, time > MIC, protein binding)
- MIC for bacteria
- Evaluate dosage levels

Population Variation



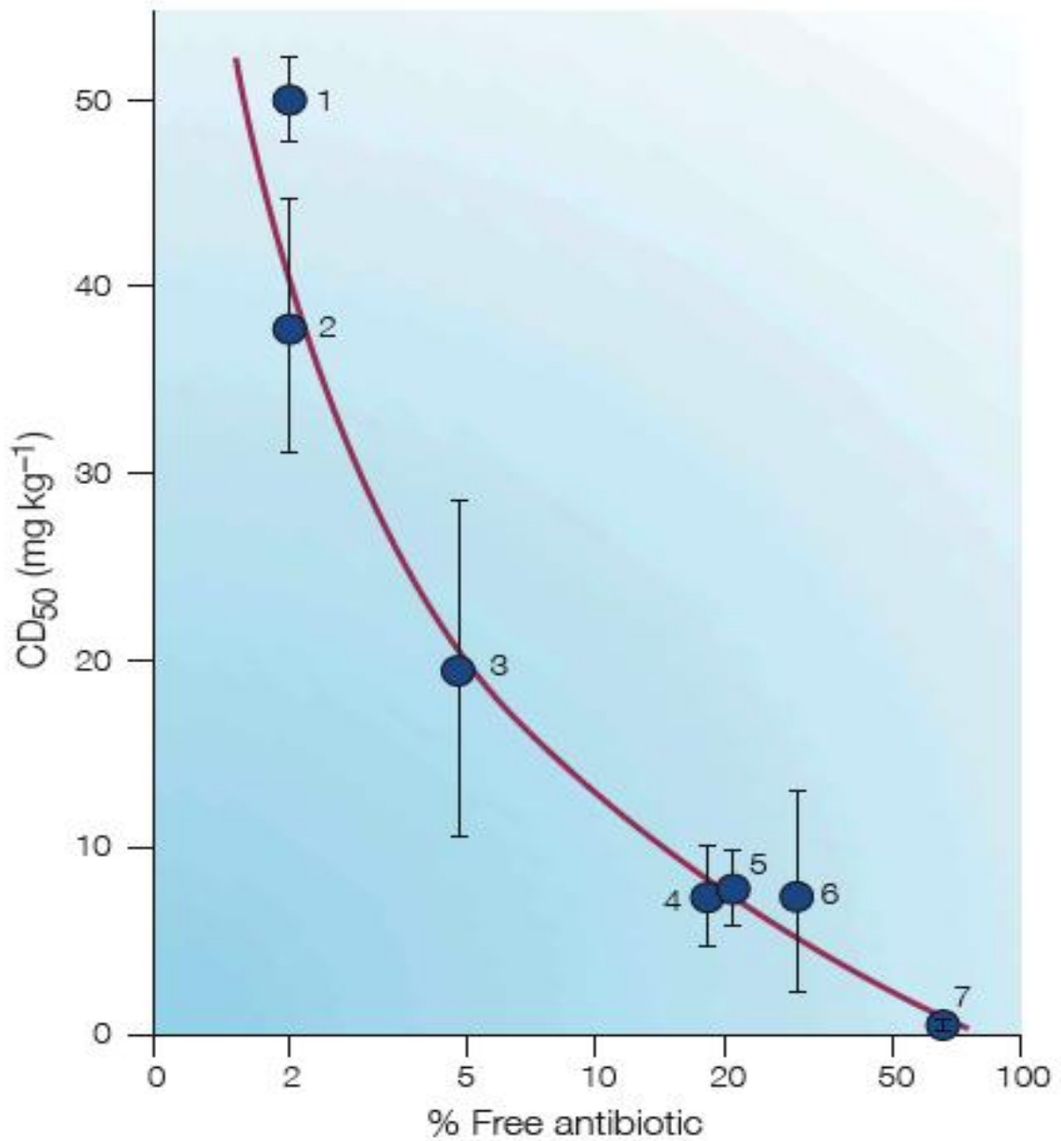
Source: Drusano, G. L. "Pharmacokinetics and Pharmacodynamics of Antimicrobials." *Clinical Infectious Diseases* 45, no. Supplement 1 (2007): S89-95.

252 Patients with Community Acquired Infections

CID, 2007, 45, S89

Protein Binding

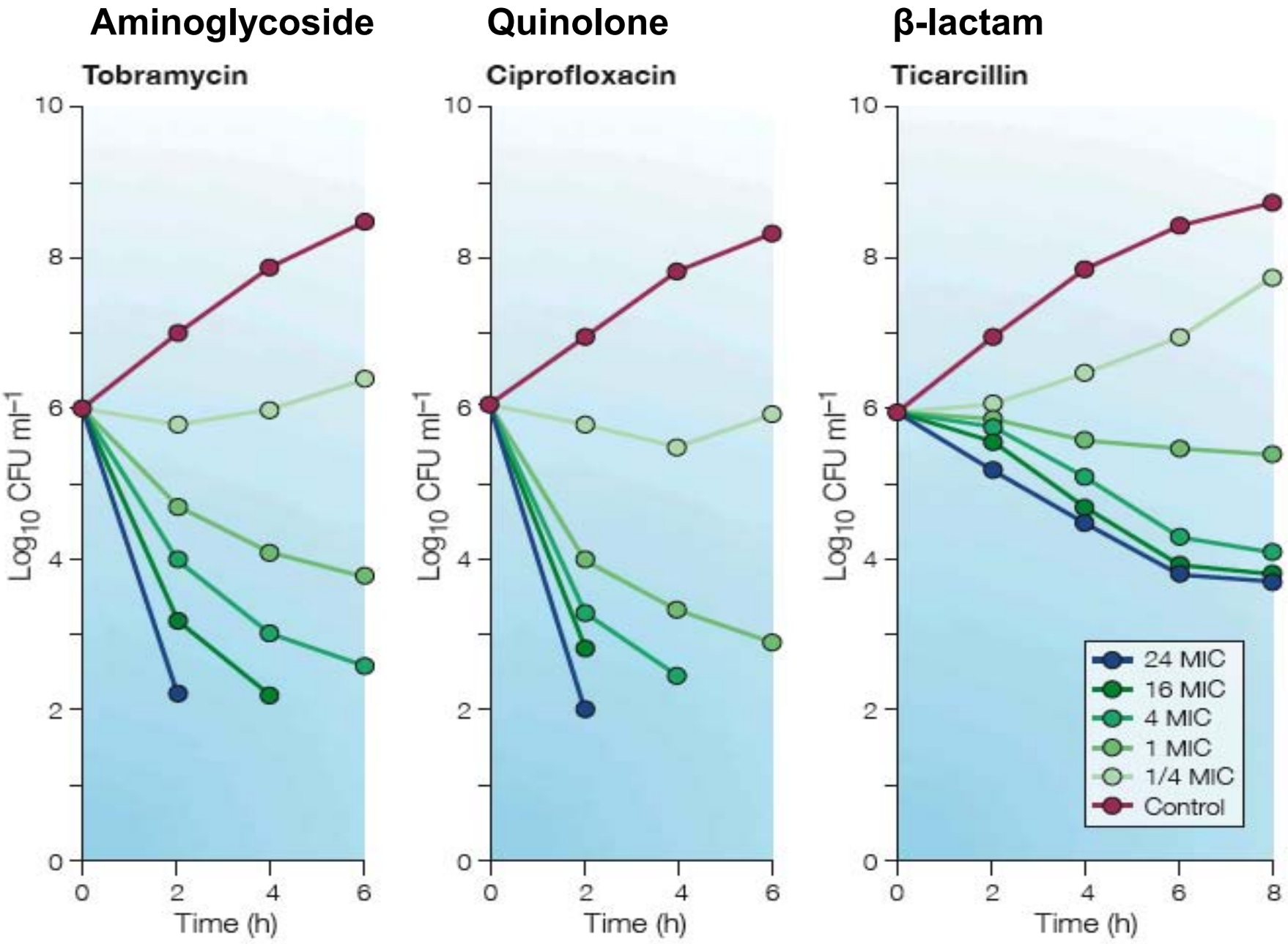
- *Staphylococcus aureus* mouse model (IP)
- 7 separate structurally similar β-lactams
- All have identical MIC
- Only free drug is pharmacologically active



Source: Drusano, George L. "Antimicrobial Pharmacodynamics: Critical Interactions of 'Bug and Drug'." *Nature Reviews Microbiology* 2, no. 4 (2004): 289-300.

Nat. Rev. Microbiol., 2004, 2, 289

Bacterial Kill Rates



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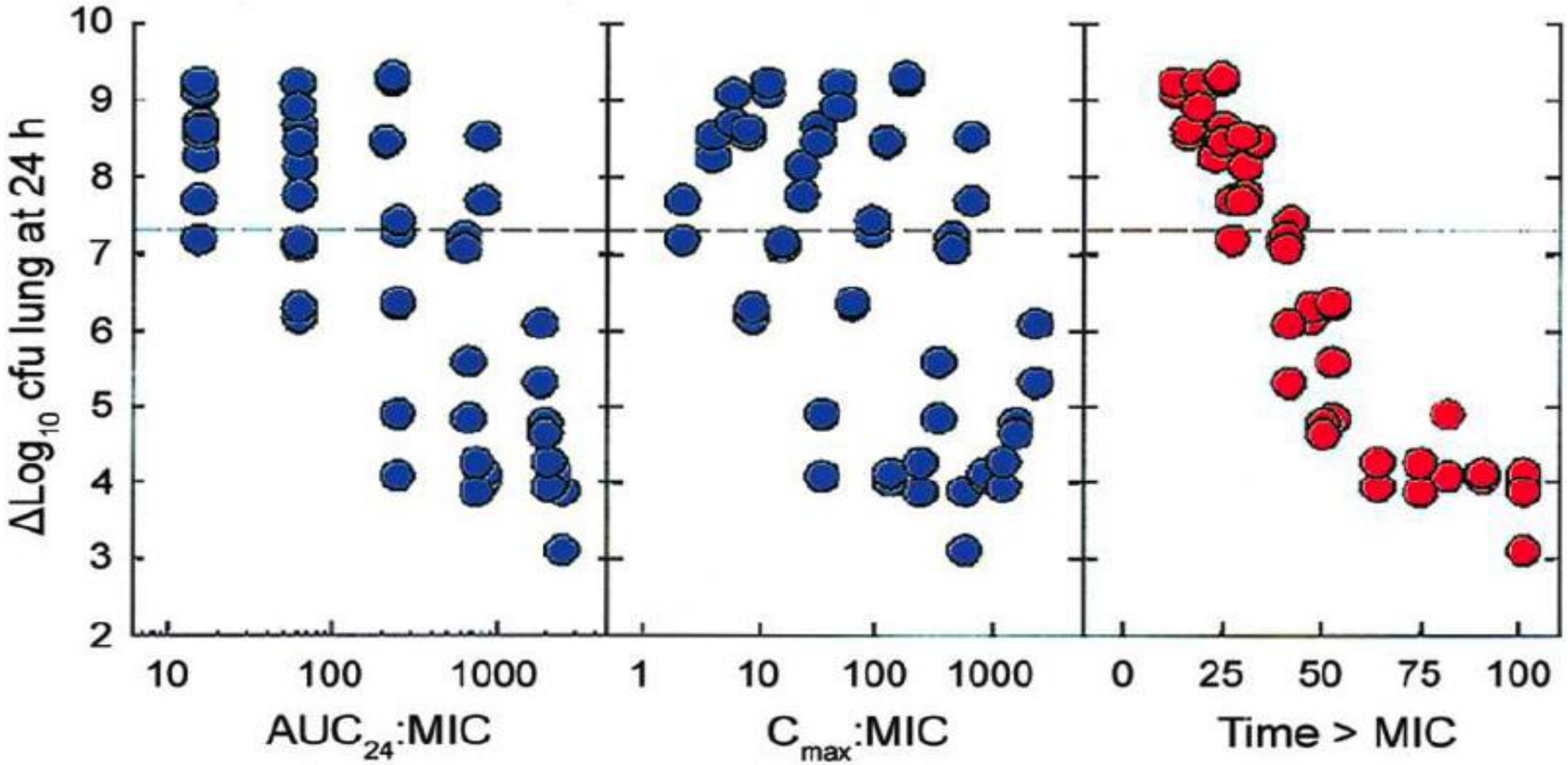
Source: Drusano, George L. "Antimicrobial Pharmacodynamics: Critical Interactions of 'Bug and Drug'." *Nature Reviews Microbiology* 2, no. 4 (2004): 289-300.

Nat. Rev. Microbiol., 2004, 2, 289

Linking Exposure to Efficacy

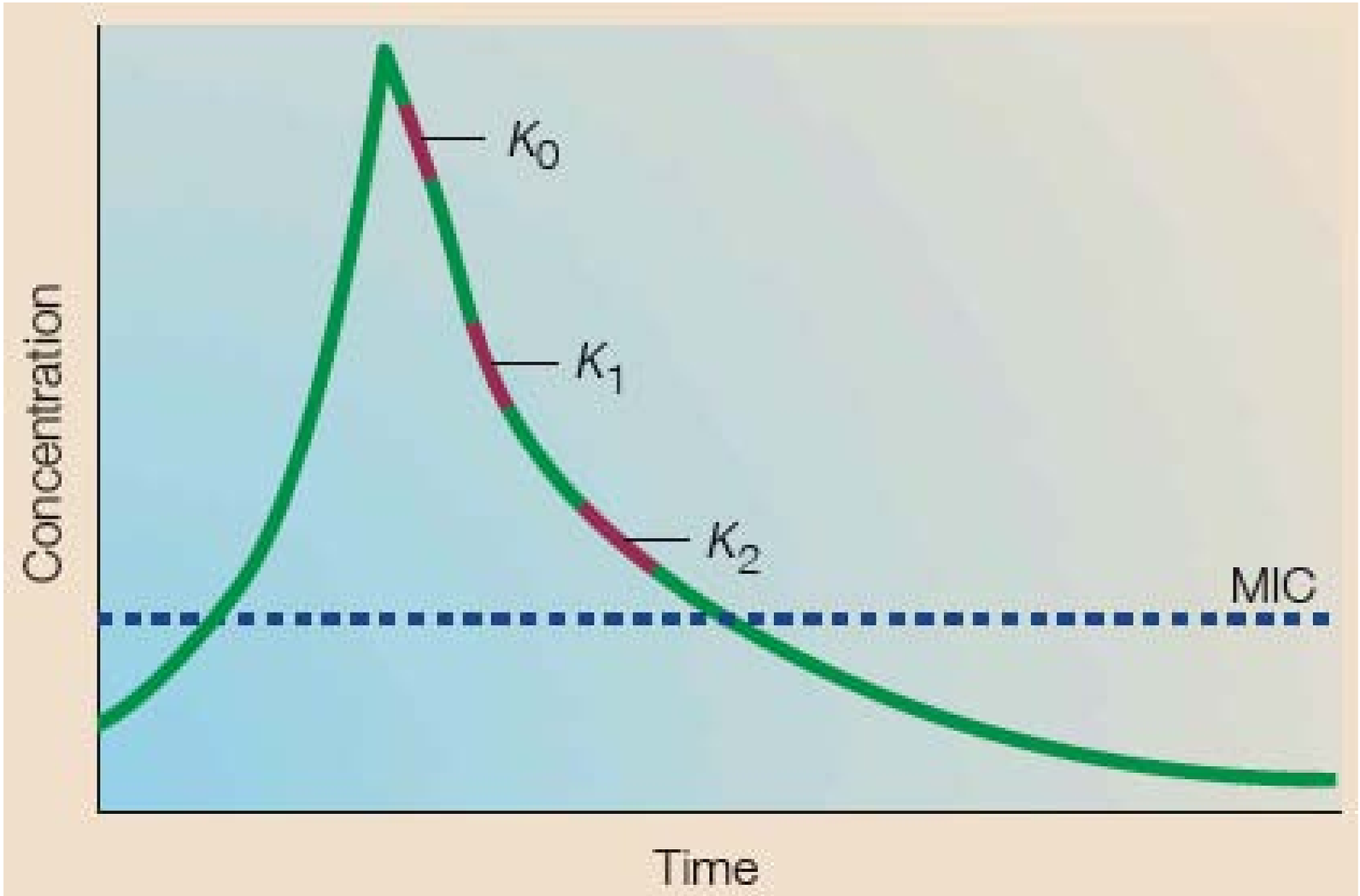
Cefotaxime β-lactam
Neutropenic Animals



Source: Drusano, G. L. "Pharmacokinetics and Pharmacodynamics of Antimicrobials." *Clinical Infectious Diseases* 45, no. Supplement 1 (2007): S89-95.

CID, 2007, 45, S89

PD-Dependence on Kill Curves



Courtesy of Macmillan Publishers Limited. Used with permission.
Source: Drusano, George L. "Antimicrobial Pharmacodynamics: Critical Interactions of 'Bug and Drug'." *Nature Reviews Microbiology* 2, no. 4 (2004): 289-300.

β-lactams: $K_0 \sim K_1 \sim K_2$

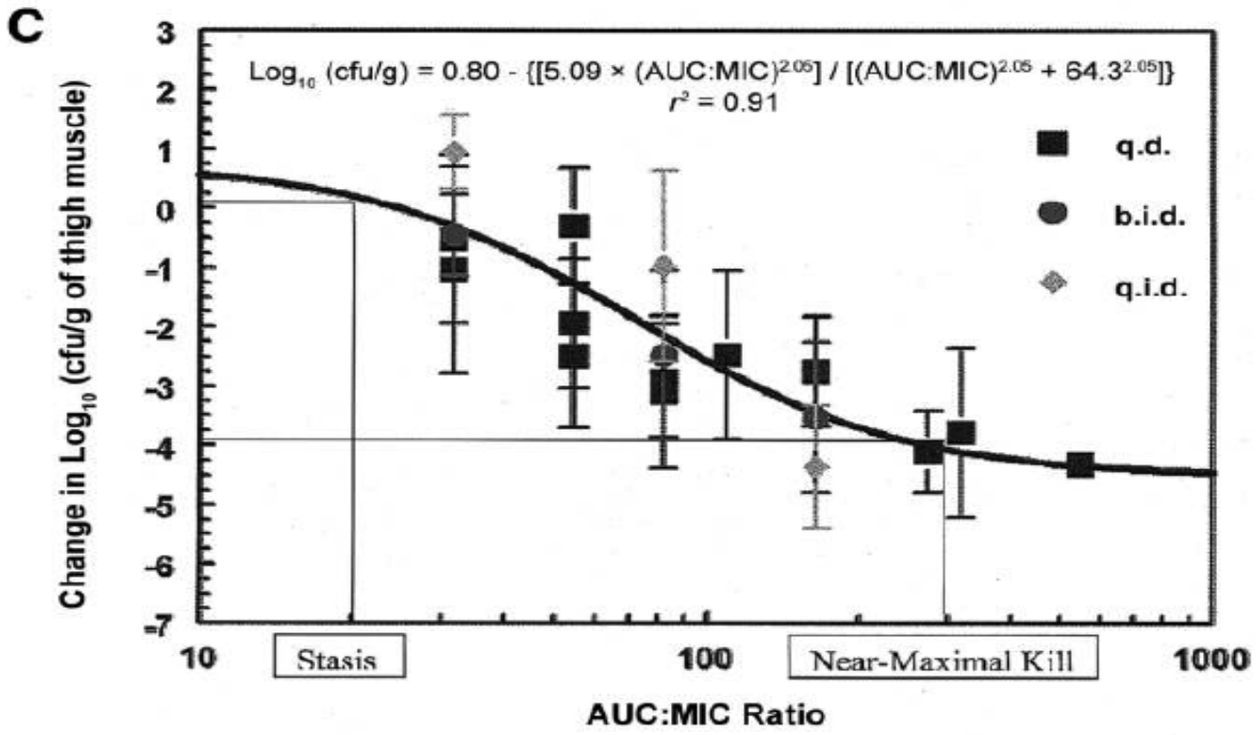
Quinolones, Aminoglycosides: $K_0 > K_1 > K_2$

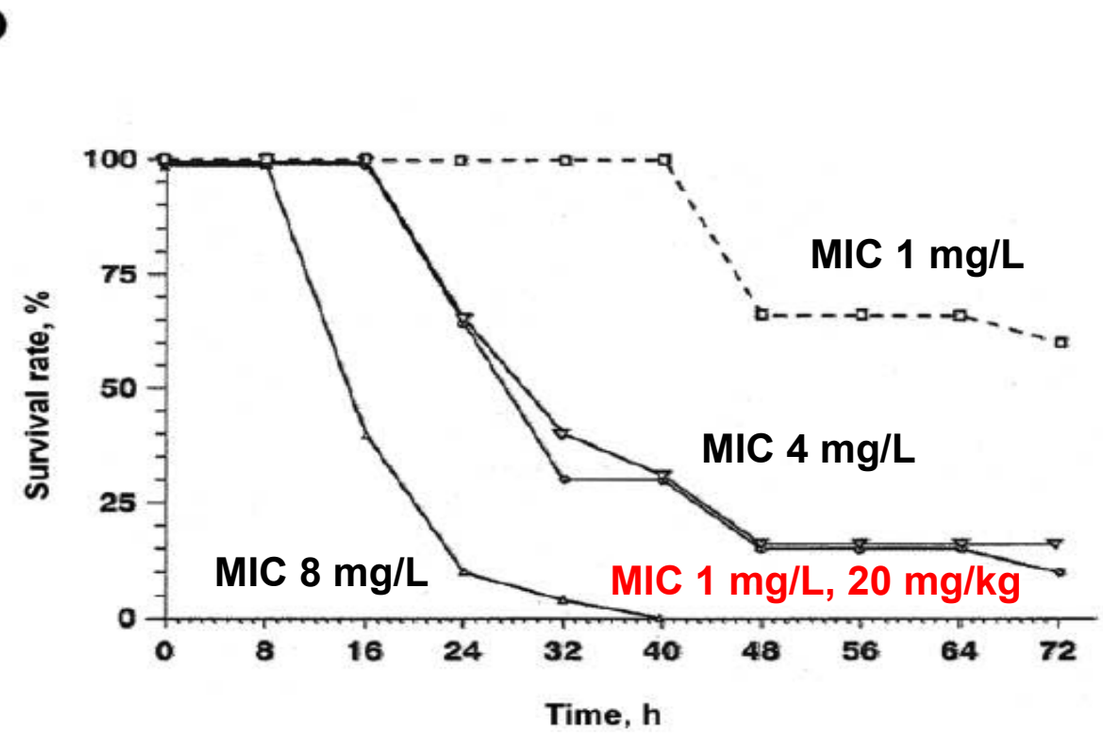
Nat. Rev. Microbiol., 2004, 2, 289

Fluoroquinolones: AUC to MIC Matters

q.d. = A dose 1x/day
b.i.d. = A/2 dose, 2x/day
q.i.d. = A/4 dose, 4x/day

80 mg/kg daily dose
lomefloxacin (fluoroquinolone)
3 strains of *Pseudomonas aeruginosa*
Neutropenic Rats





Source: Drusano, G. L. "Pharmacokinetics and Pharmacodynamics of Antimicrobials." *Clinical Infectious Diseases* 45, no. Supplement 1 (2007): S89-95.

Lomefloxacin (fluoroquinolone)
S. pneumoniae
Model: Mouse thigh infection

CID, 2007, 45, S89

Emergence of Resistance

- Heterogeneous cell populations
  - Hypermutators and persisters
- High mutation rate
  - Mutations at 10^{-8} to 10^{-6} genes per generation
- Rapid growth rate
  - Double approximately every 30 minutes
- Readily transfer genetic material
- Improper treatment selects for resistant cultures

Persister Phenotype

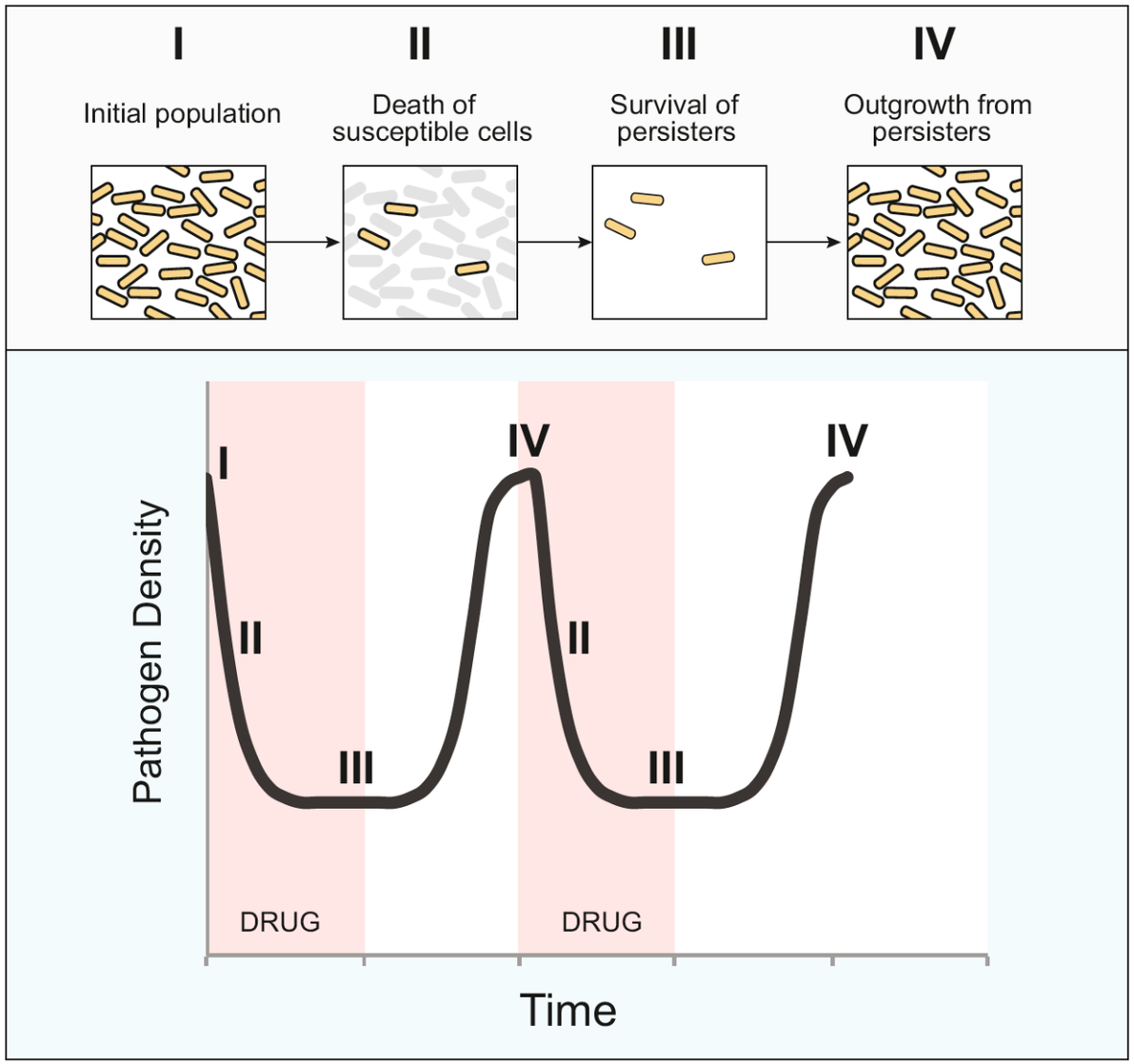


Figure 1. Drug Persistence and Recurrent Infection

Source: Cohen, Nadia R., Michael A. Lobritz, et al. "Microbial Persistence and the Road to Drug Resistance." *Cell Host & Microbe* 13, no. 6 (2013): 632-42.

- Distinct from resistance
  - No expansion in presence of antibiotic
  - Population growth upon removal
- Nonhereditary phenotype
- Problematic for “compromised” individuals of the population

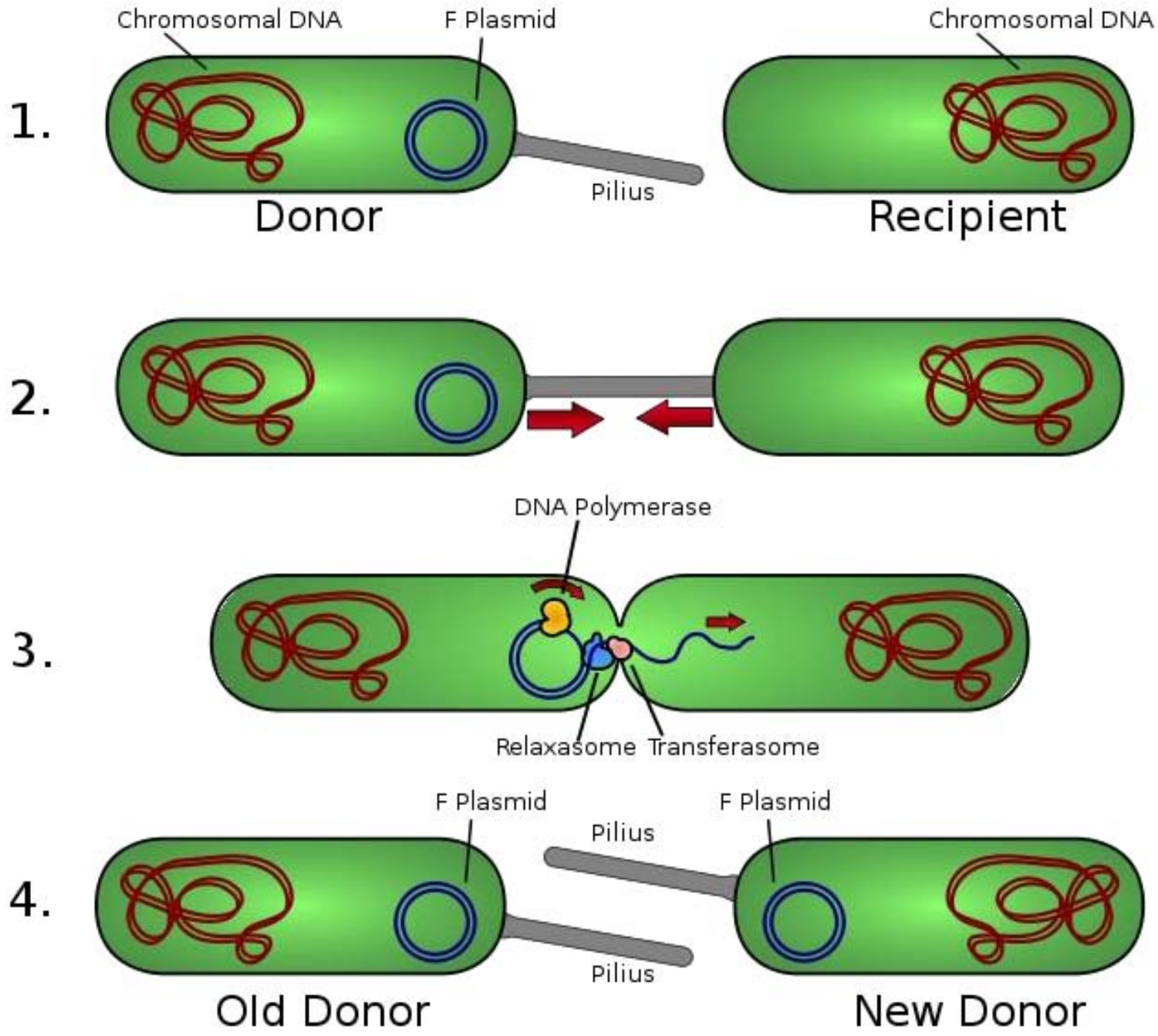
Cell Host & Microbe, 2013, 13, 632

Genetic Variation

- Point mutations
  - Vertical transmission (germ line)
- Plasmid born (conjugation)
  - Horizontal gene transfer
- Acquire environmental DNA (transformation)
  - Horizontal transmission
- Virus/bacteriophage (transduction)
  - Horizontal transmission

Conjugation

- Horizontal transfer (horizontal gene transfer)
- Plasmid can contain multiple factors that render resistance
- Can be passed between different species and genus



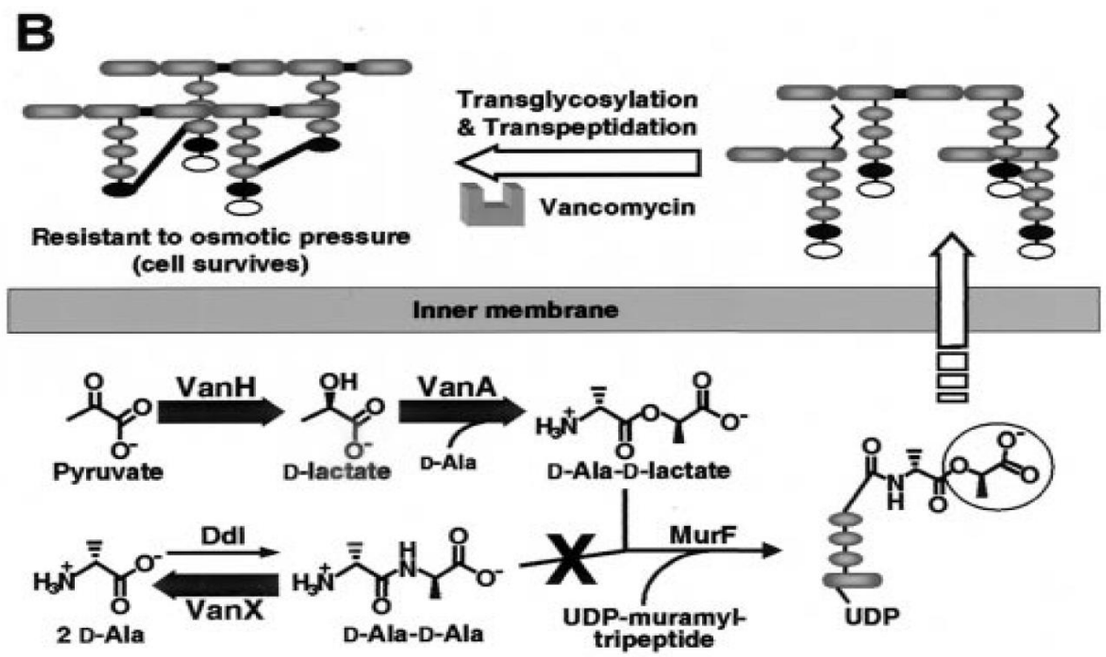
Courtesy of Michael David Jones on wikipedia. Used with permission.

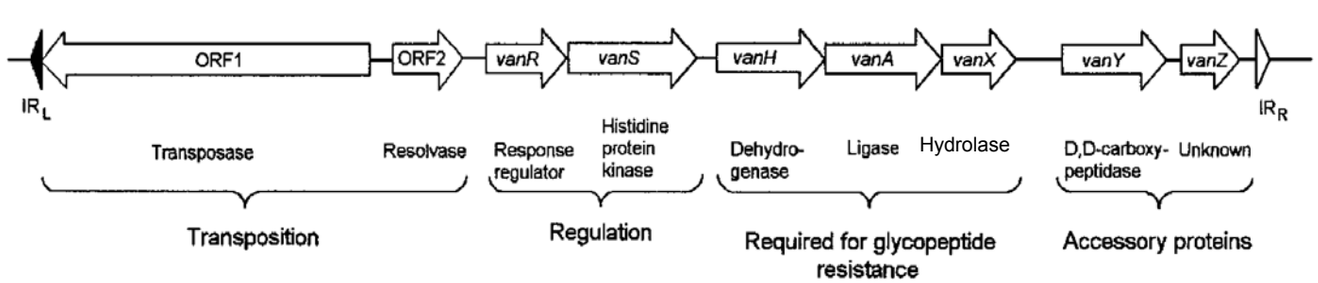
Wikipedia

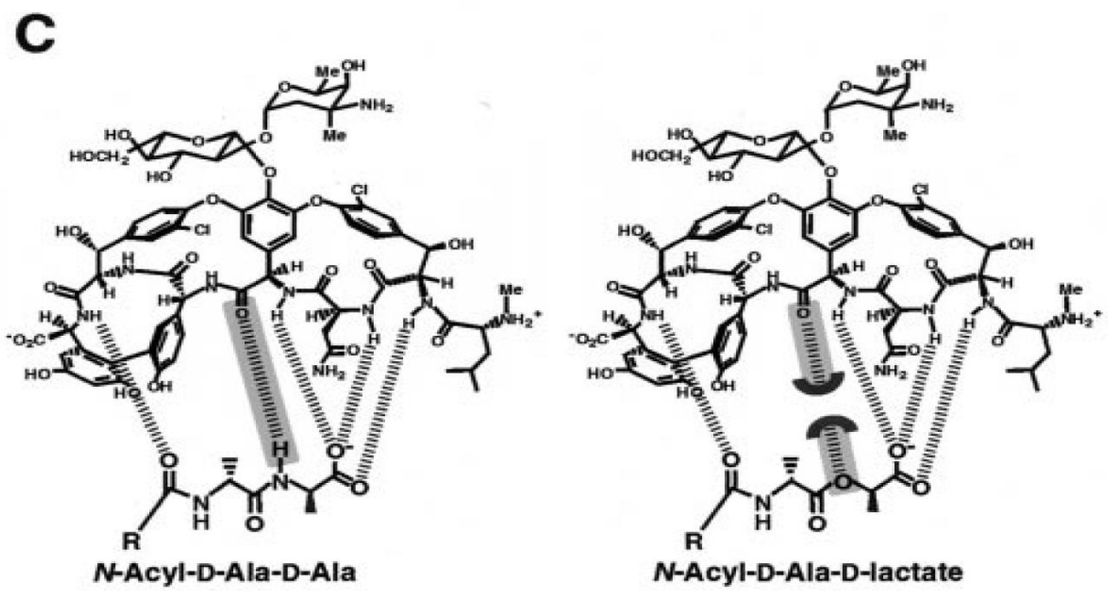
Common Resistance Mechanisms

- Metabolic enzymes
  - β-Lactamase
  - Esterase
  - Acetyltransferase
- Efflux pumps
  - Reduce concentration of drug
- Mutations in antibiotic targeted proteins
  - Topo II

Vancomycin Resistance







- 9 Genes on a transposon
- All genes can hop in and out
- VanS and VanR are regulatory genes that are only switched on in the presence of Vancomycin

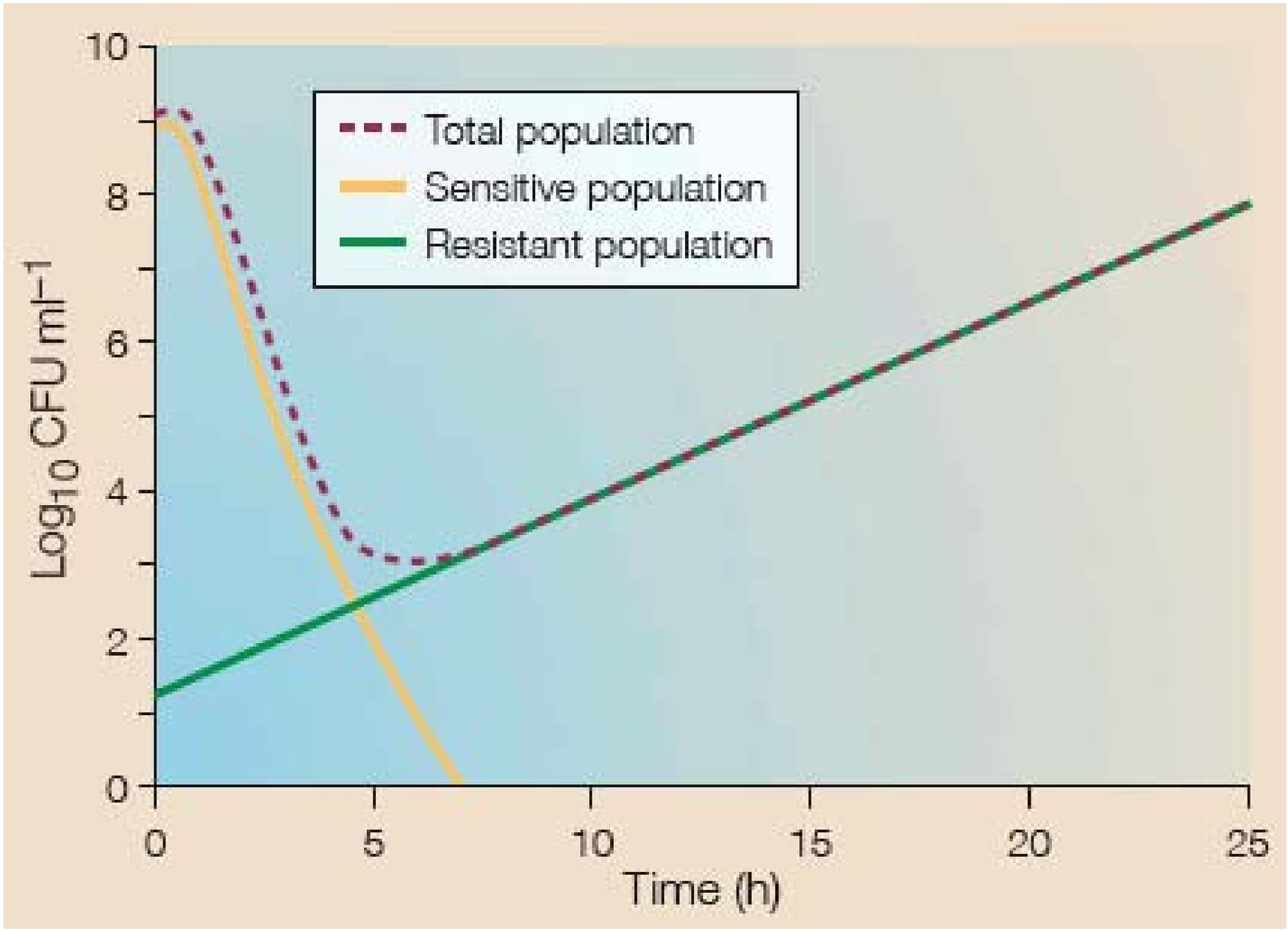
Courtesy of the National Academy of Sciences. Used with permission.
Source: Lessard, Ivan AD, and Christopher T. Walsh. "VanX, A Bacterial D-alanyl-D-alanine Dipeptidase: Resistance, Immunity, or Survival Function?" *Proceedings of the National Academy of Sciences* 96, no. 20 (1999): 11028-32.

PNAS, 1999, 96, 289

Hypermutator Phenotype

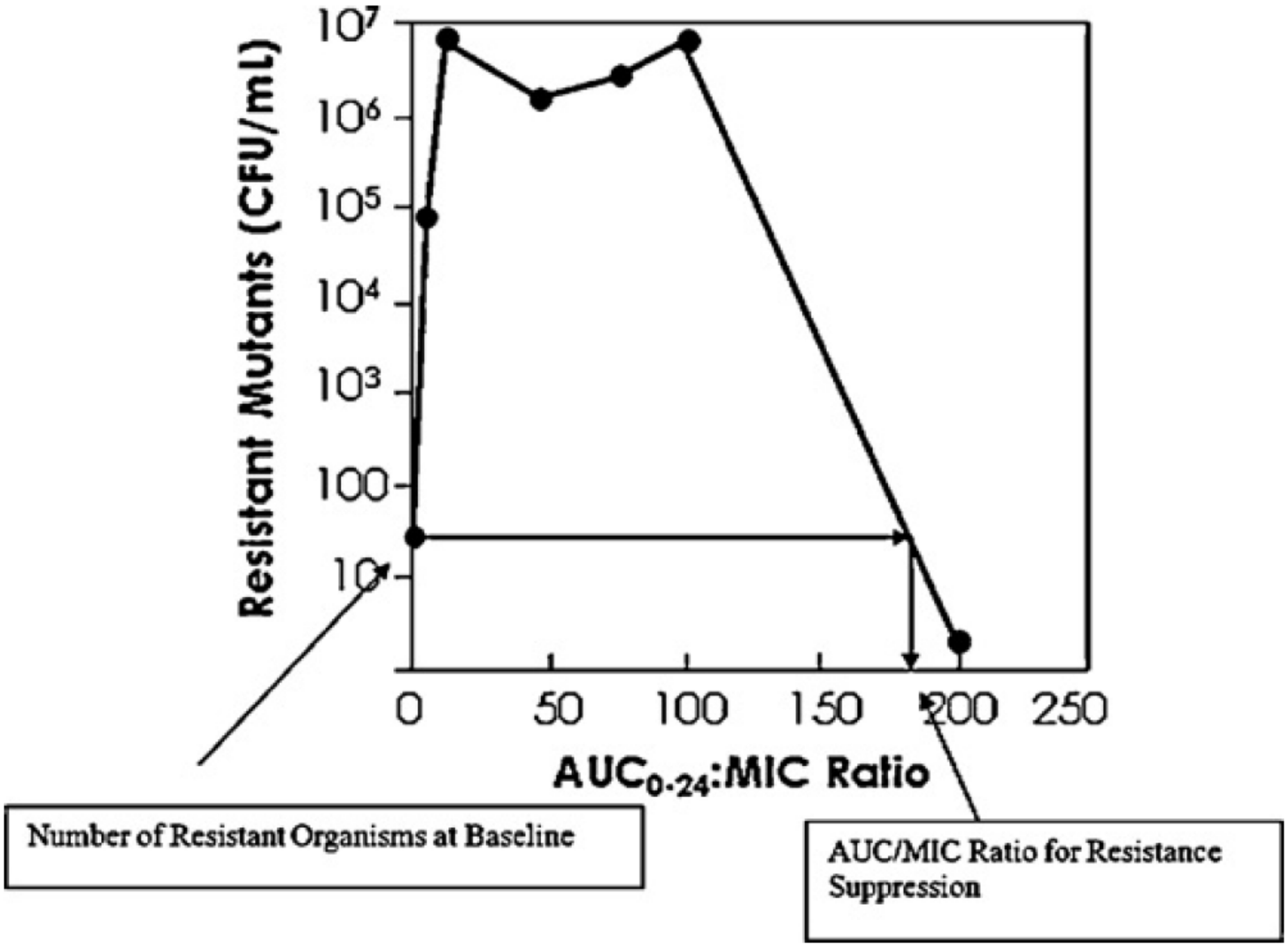
- In absence of horizontal transfer, the only possible resistance mechanism is mutation
- Mutations in DNA repair mechanisms lead to increased rates of germ line mutations
- Accelerated evolution via promiscuous repair/recombination and rapid duplication

Selective Pressure



Nat. Rev. Microbiol., 2004, 2, 289

Amplification of Resistance



Source: Mouton, Johan W., Paul G. Ambrose, et al. "Conserving Antibiotics for the Future: New Ways to use Old and New Drugs from a Pharmacokinetic and Pharmacodynamic Perspective." *Drug Resistance Updates* 14, no. 2 (2011): 107-17.

Drug Resistance Updates, 2011, 14, 107

Dosing Impacts Emergence of Resistance

- Size matters
  - Larger the bacterial load, the more likely that resistant populations exist
- Rapid and more intense the treatment the better (in general)
  - Minimize time for bacteria to mutate or transfer resistance
- Granulocytes (innate immune system) clear bacteria at appreciable rates
  - Co-dependence on antibiotics to limit growth and impact population size
- Evaluation of PK/PD antimicrobial parameters are on a case-by-case basis. More work needs to be done in vivo
- Predictive tools for infection type and virulence are needed

20.201 Mechanisms of Drug Actions

Fall 2013