Impact of the Protein Data Bank on Drug Development

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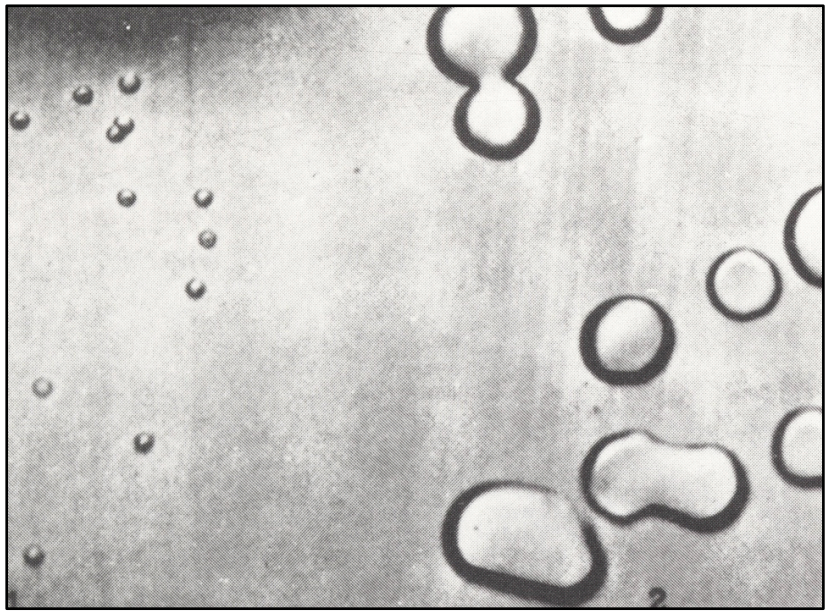
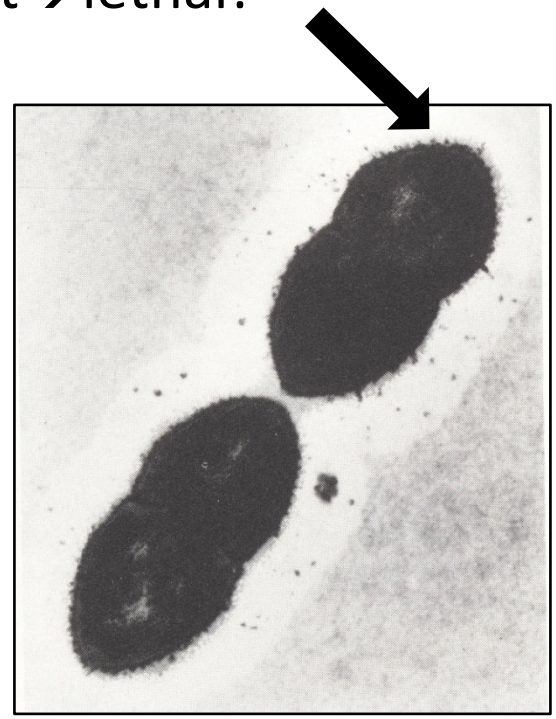
Outline

- DNA/RNA/Protein and the Genomics Revolution
- Drug Discovery Case Studies from the PDB
  - Treatment of Bacterial Infections
  - Treatment of a Blood Cancer
- Learn more about drugs using the PDB
  - More small molecules
  - Therapeutic antibodies

DNA is the Transforming Principle

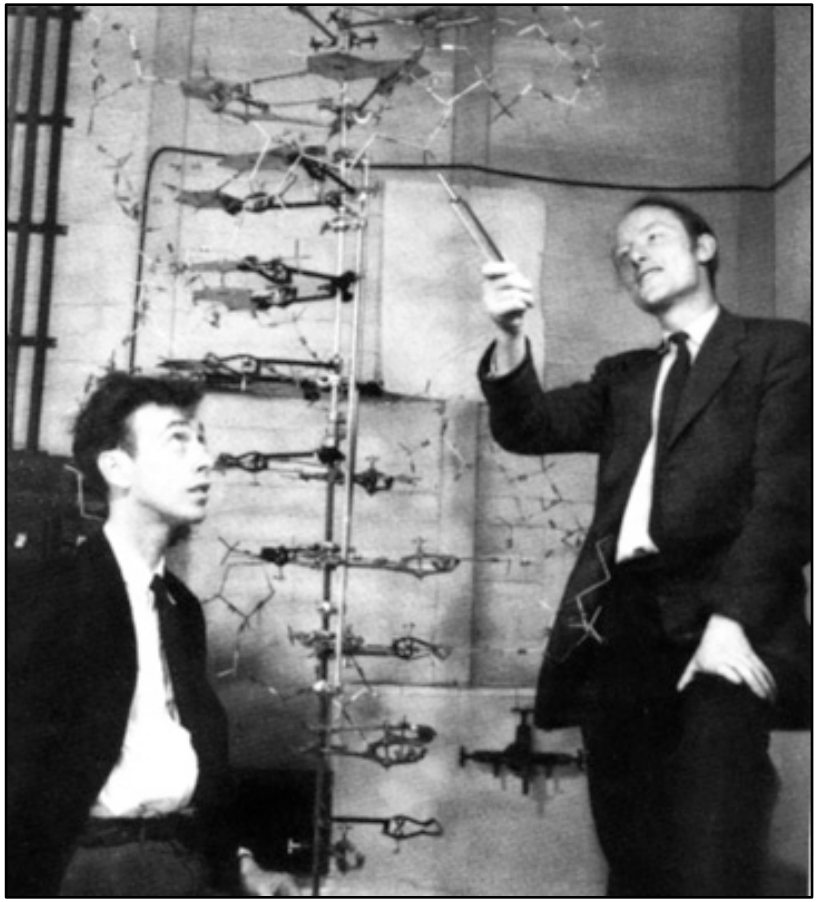
- Avery, McLeod, and McCarty experiment (1944)
- *Streptococcus pneumoniae* exchange DNA
  - Small colonies=no polysaccharide coat→non-pathogenic
  - Large colonies=polysaccharide coat→lethal!
  - Extra genes make the difference



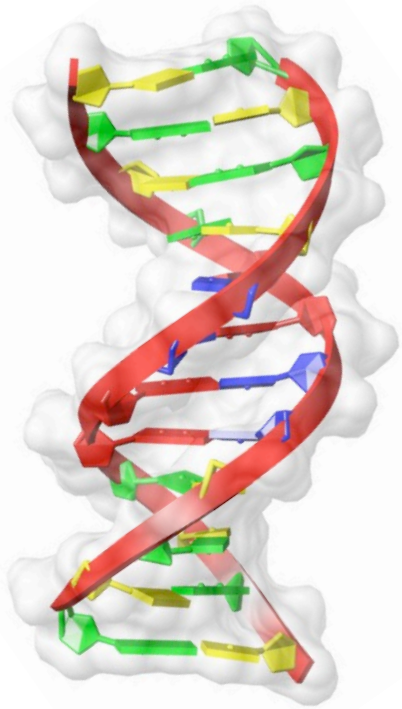


Structure of the DNA Double Helix

- Watson-Crick Model

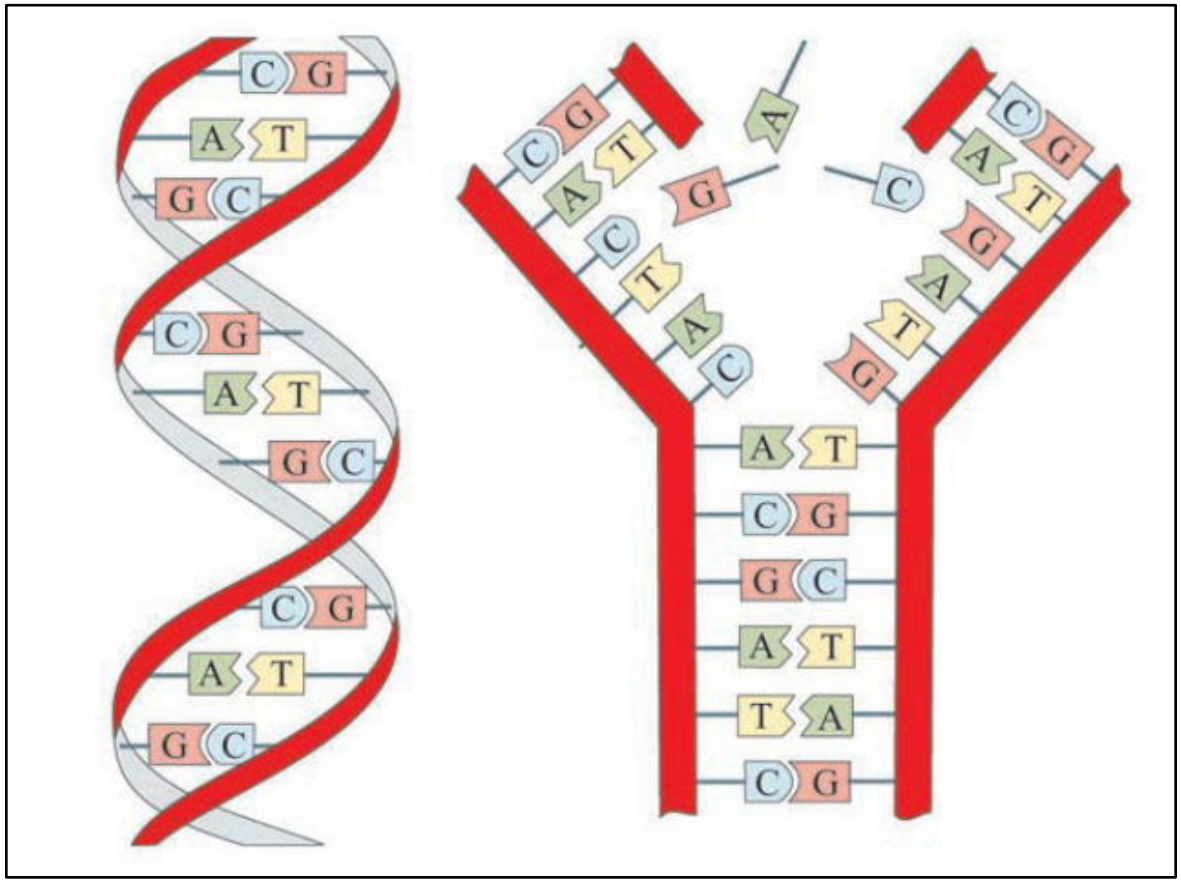


- PDB is a rich source of atomic resolution DNA structures (1bna)



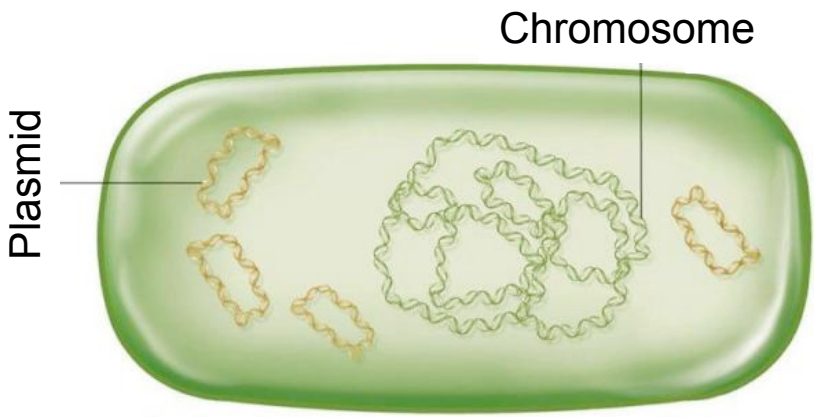
Base Pairing → Double Helix
Double Helix Structure → Function

- Adenine-Thymine (A-T)
- Guanine-Cytosine (G-C)

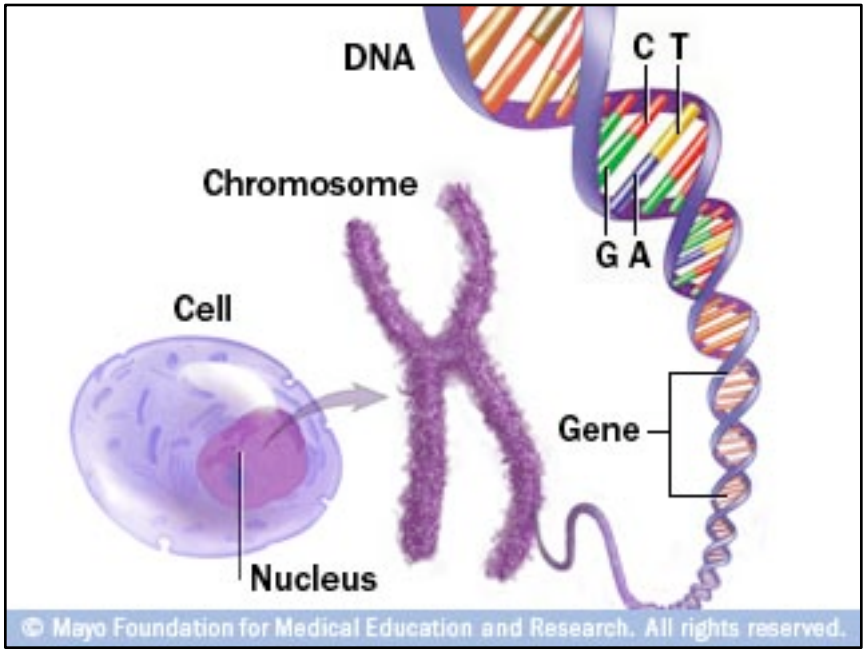


Organization of DNA
Circular and Linear Chromosomes

- Bacterial DNA is Circular
- Chromosomes hold 1,000s of genes
- Plasmids hold just a few genes and move between cells

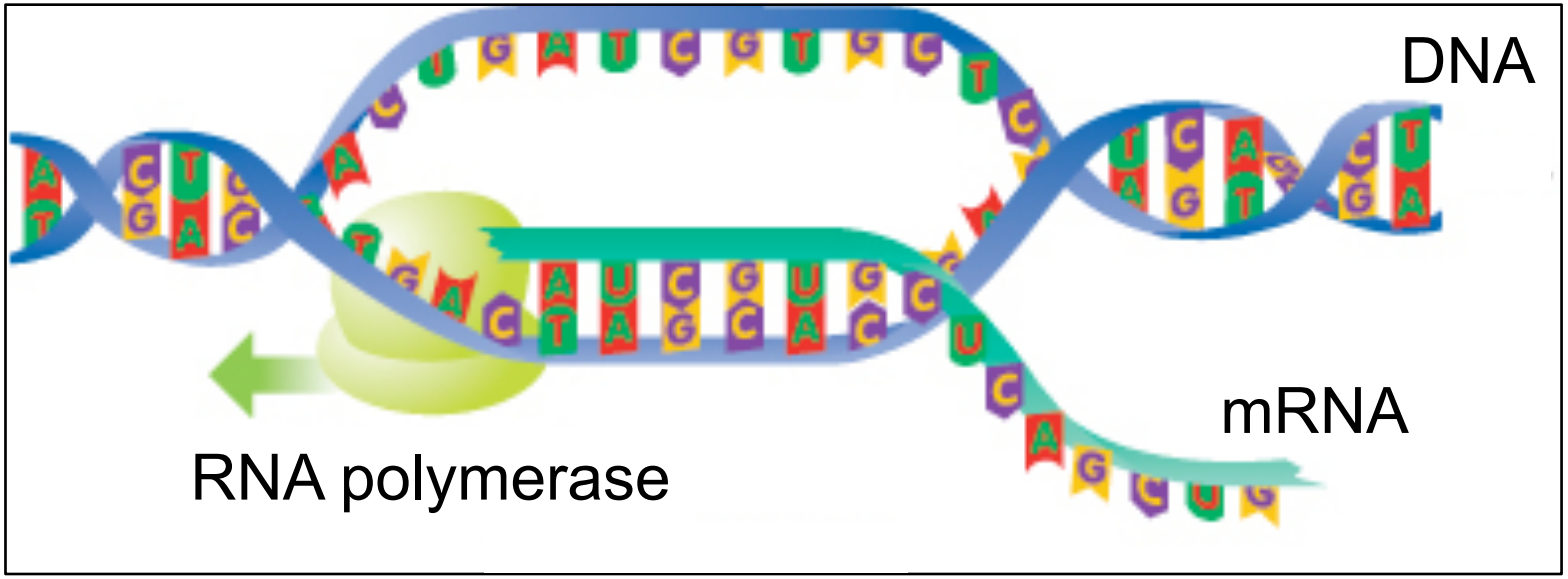


- Human DNA is Linear
- 46 Chromosomes hold ~20,000 genes



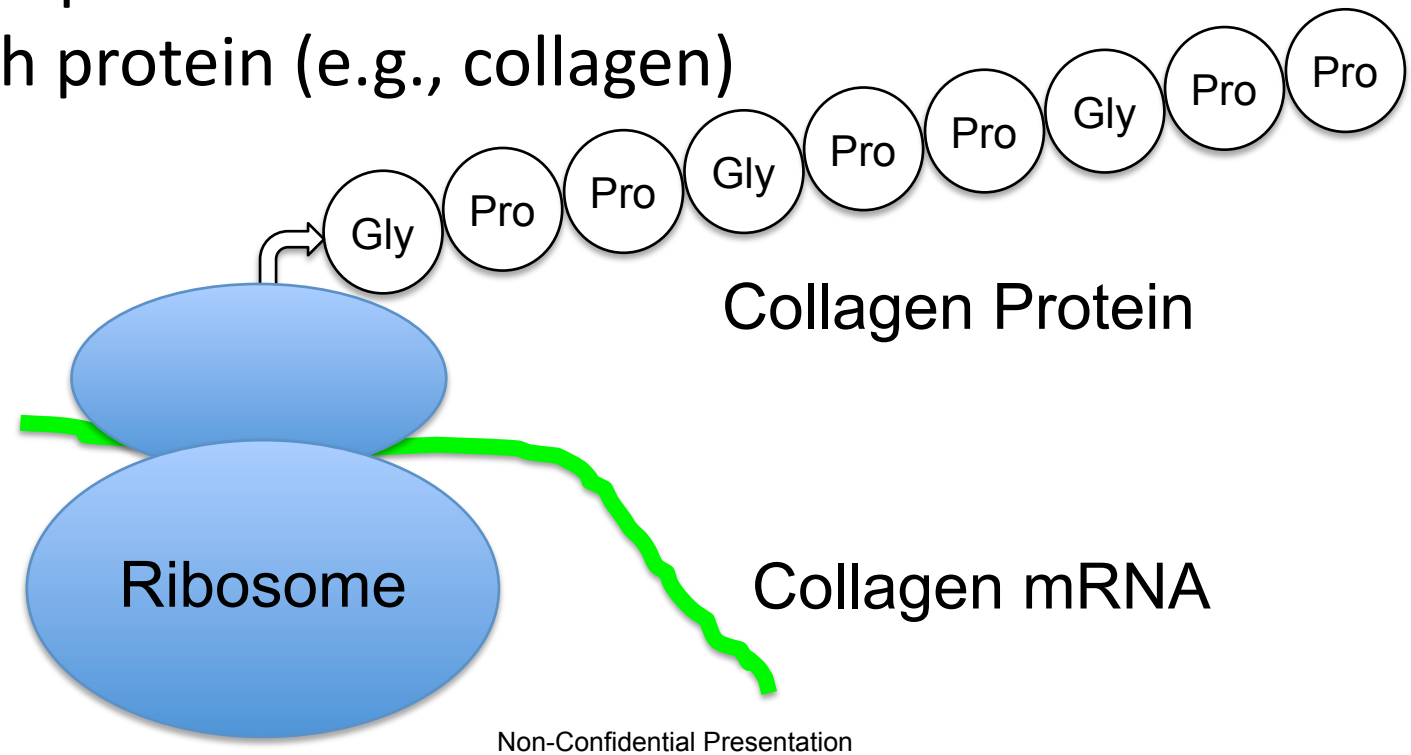
DNA makes RNA makes Protein I

- Transcription of DNA → messenger RNA
- DNA is the "blueprint" that passes genetic information from one generation to the next
- DNA encoded genes determine <u>genotype</u>



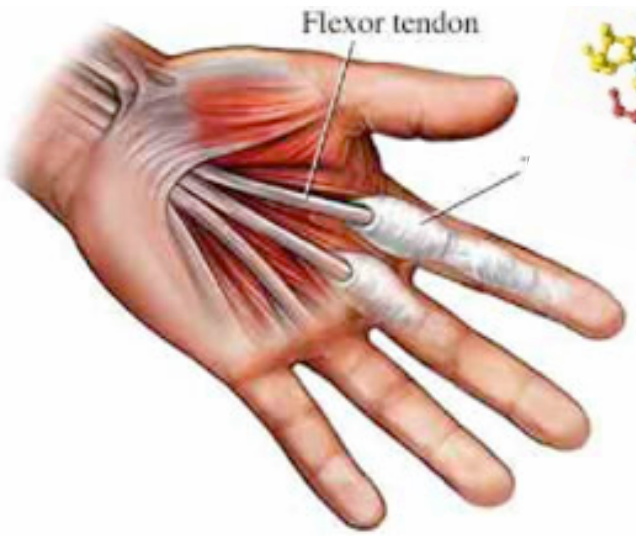
DNA makes RNA makes Proteins II

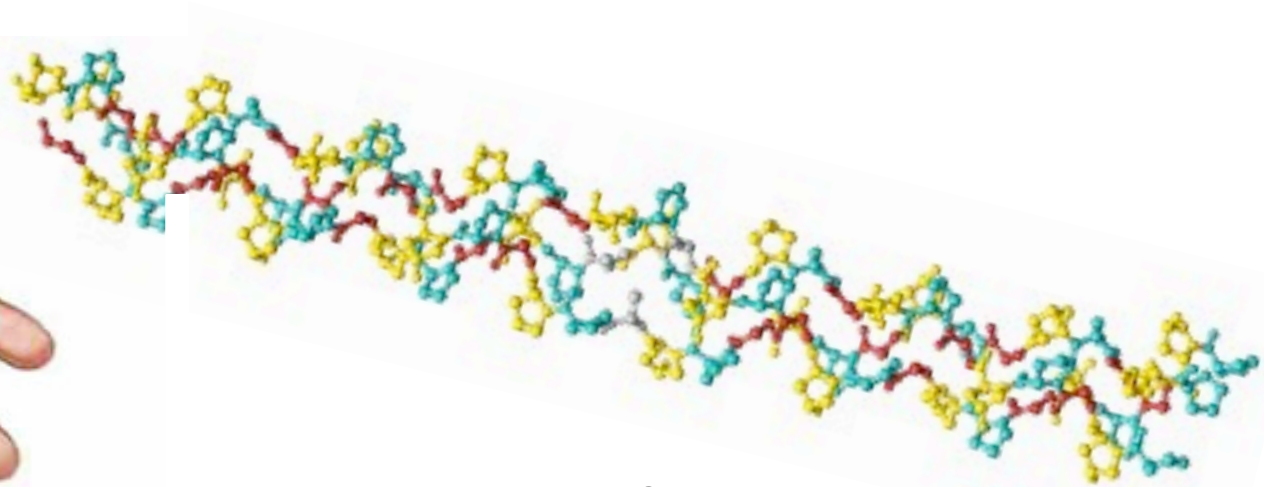
- Translation of messenger RNA → Protein (Phenotype)
- mRNA is a "working drawing" extracted from the "blueprint" that instructs the Ribosome how to make each protein (e.g., collagen)



DNA makes RNA makes Proteins III

- Proteins make up the building blocks of cells and the "machines" that support life.
- Different proteins → Different types of tissues
- Collagen triple helix molecular rope builds tendons

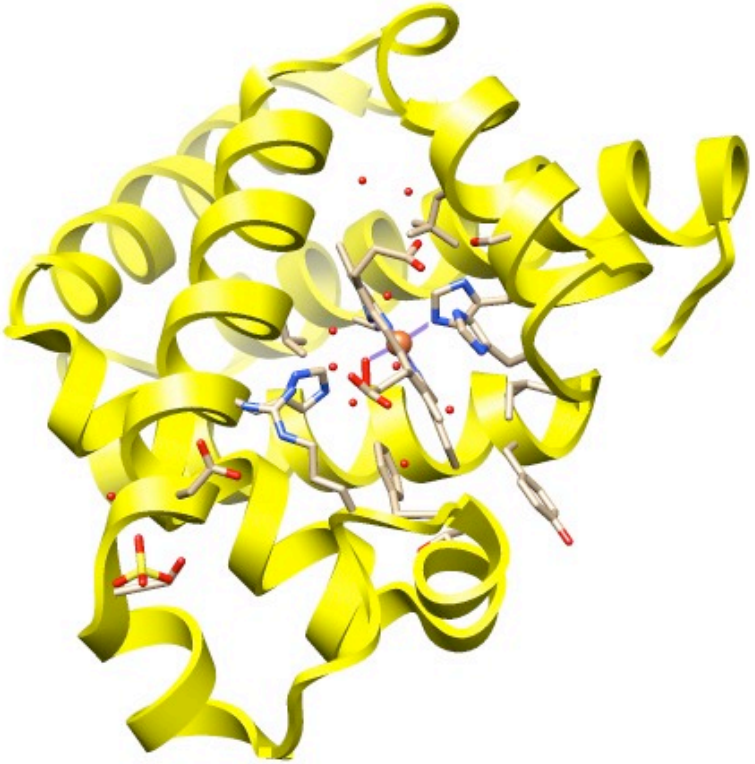




Collagen triple helix
PDB ID 1cag

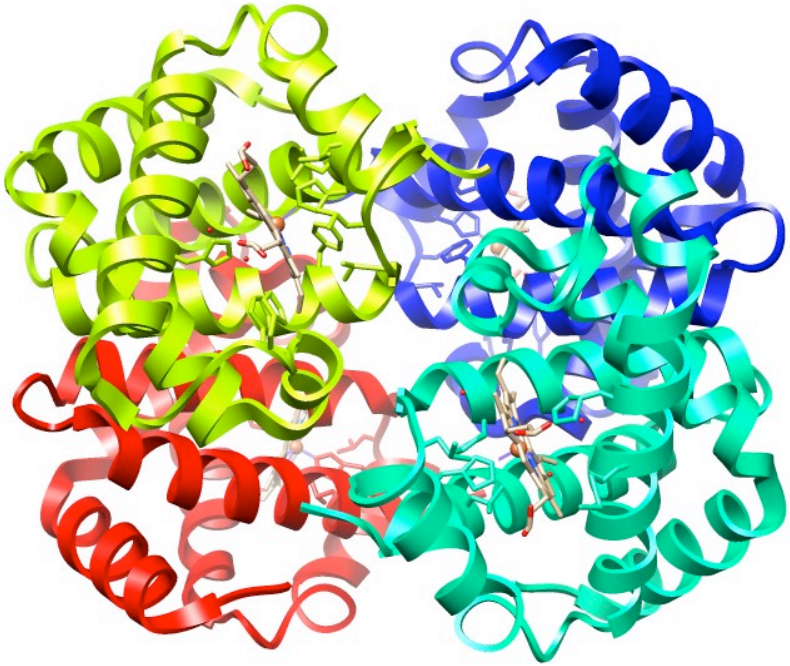
Protein Structure → Biological Function

- Myoglobin stores oxygen in muscles



PDB ID 1mbo

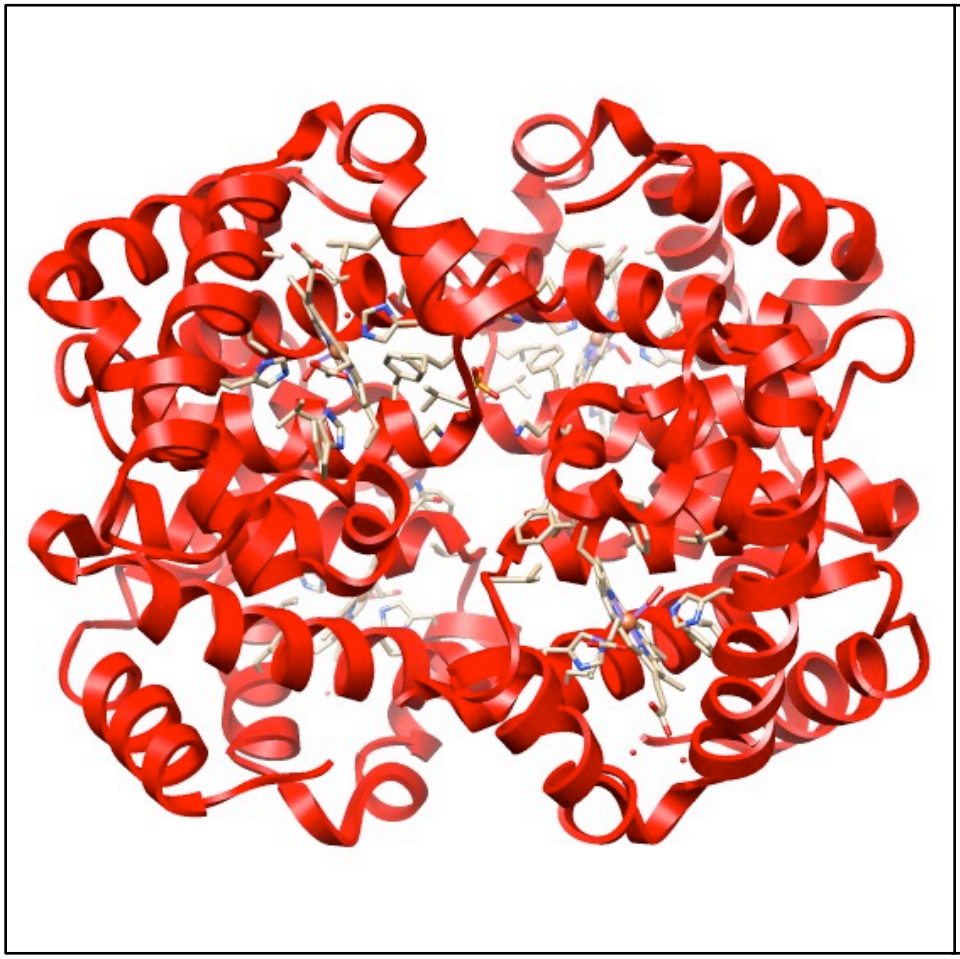
- Hemoglobin found in red blood cells moves oxygen (lung→tissues)

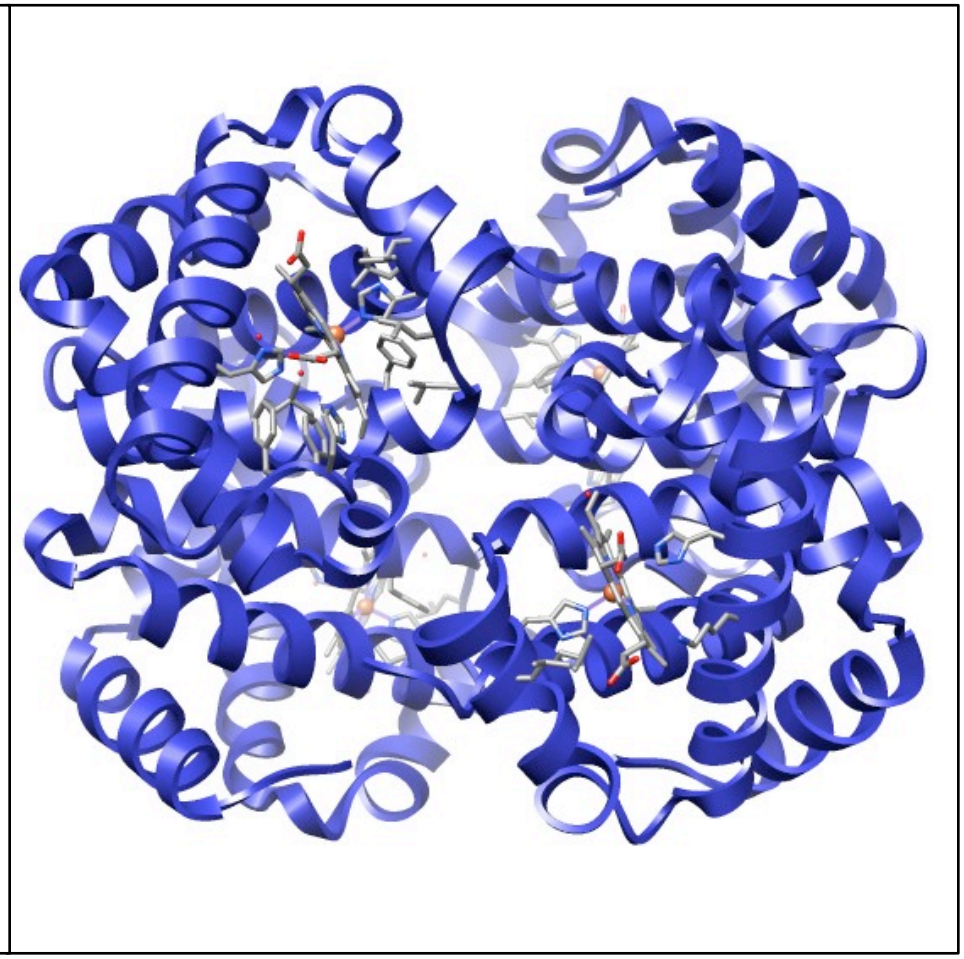


PDB ID 4hhb

Hemoglobin (Hb) in Action

Oxy Hb in Arteries/Deoxy Hb in Veins





PDB IDs 1hho and 2hhb

Genome Sequencing → Molecular Medicine

- Human Genome
  - Chromosomes 1-22
  - X and Y Chromosome
  - ~20,000 Genes
  - Mitochondrial DNA
    - 13 genes
  - 99.7% Identical to Neanderthal
  - 98.8% Identical to Chimpanzee

  - Today's Cost<JP¥100,000

Two Routes→New Drugs

- <u>Phenotypic Discovery</u>
  - Study how cells and/or animals respond to small molecules (<500 Daltons)
  - Small molecules can come from natural products, chemical libraries, and combinatorial chemistry
  - Example: Penicillin
- <u>Targeted Discovery</u>
  - Study how individual protein targets respond biochemically to these same small molecules
  - Example: Gleevec

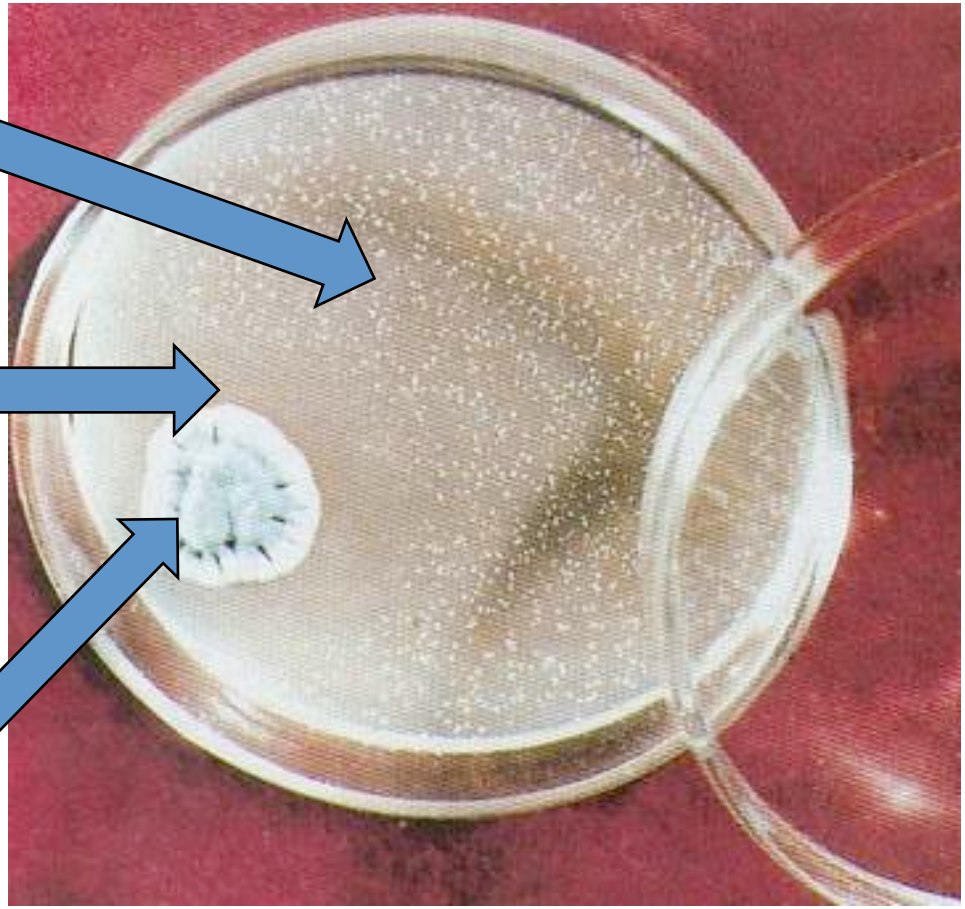
"Accidental" Discovery of Penicillin

Fleming (1928)

Staphylococcus aureus Colonies

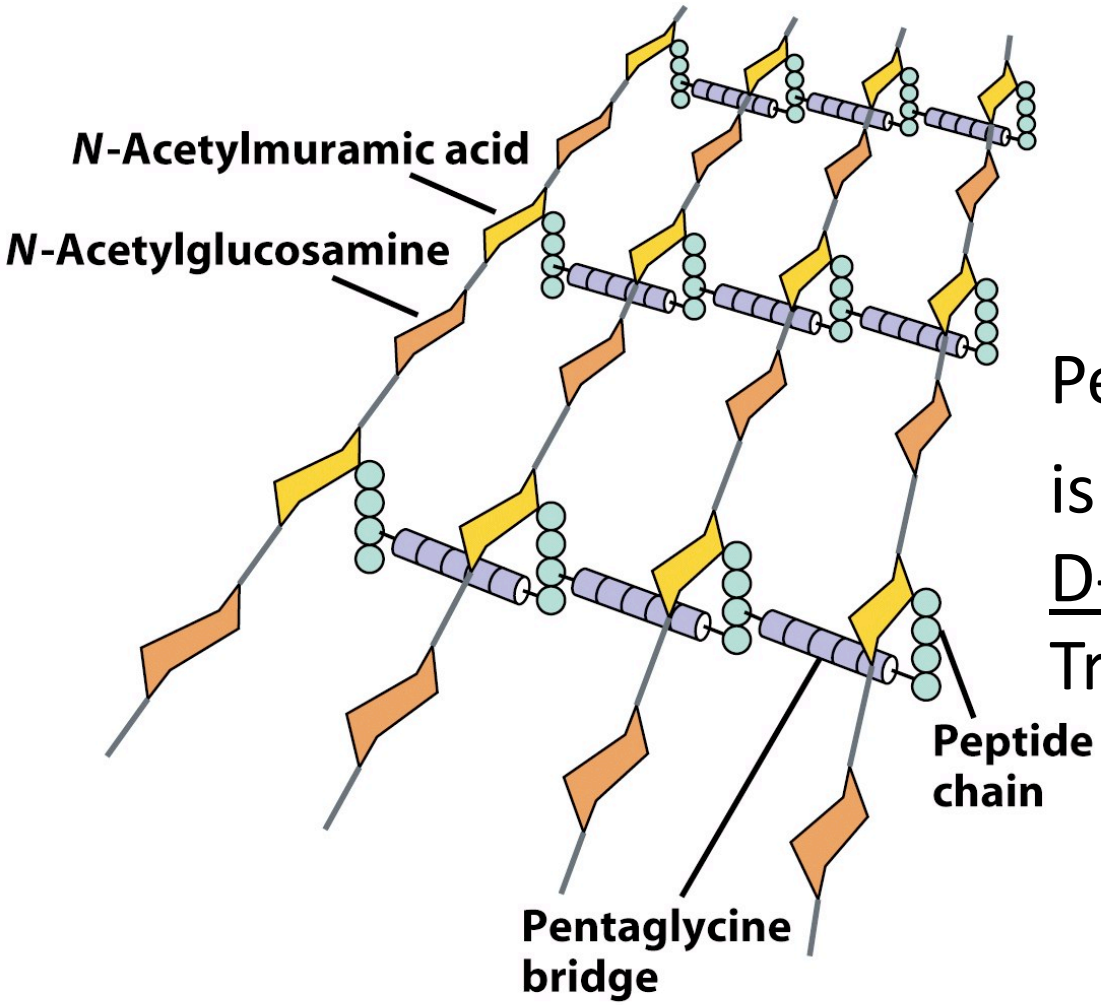
"No Growth" Halo due to "Antibiotic" Secretion by Fungus

Penicillium notatum Fungus Colony



"Nature Favors the Prepared Mind" Louis Pasteur

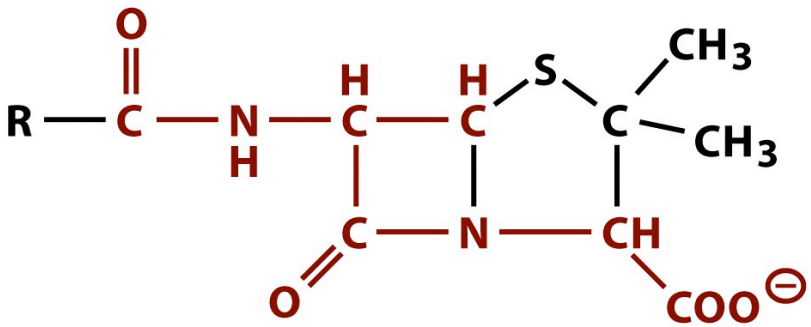
Bacterial Cell Wall Peptidoglycan Layer: Molecular Chain Mail/Kusari Armor

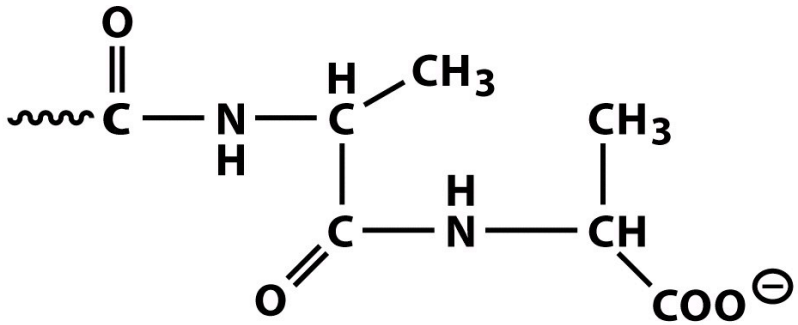


Peptide Chain X-link is assembled by a <u>D-Ala-D-Ala-</u> Transpeptidase

Penicillin is a Molecular Mimic of D-Ala-D-Ala Required to make the Kusari Cross-Link

- Fleming discovered Penicillin "accidentally" by <u>phenotypic</u> screening
- Fleming purified the natural substance (Penicillin) from the fungus
- Penicillin is a molecular mimic of D-Ala-D-Ala
- Comparison of Penicillin with D-Ala-D-Ala

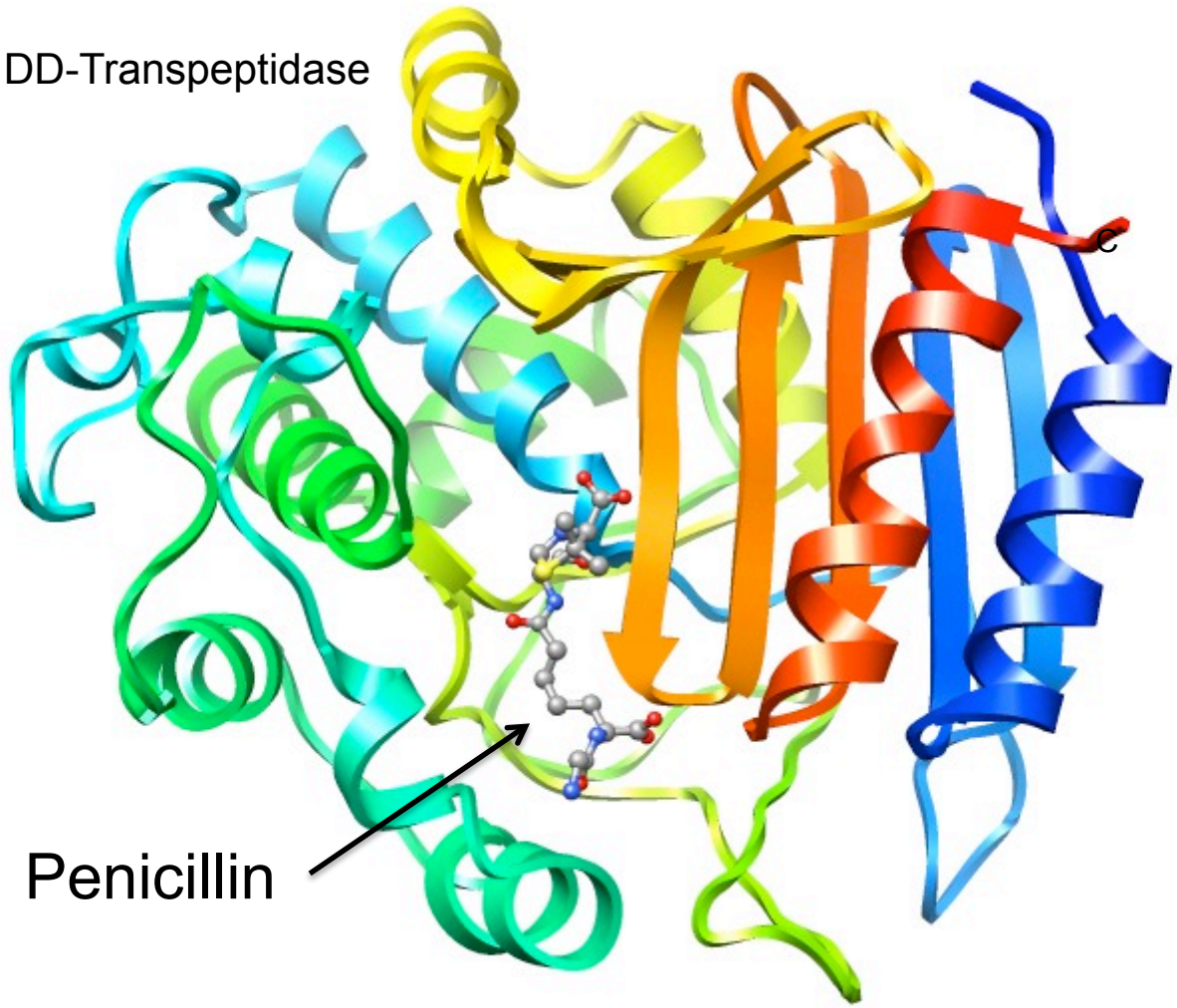




D-Ala – D-Ala

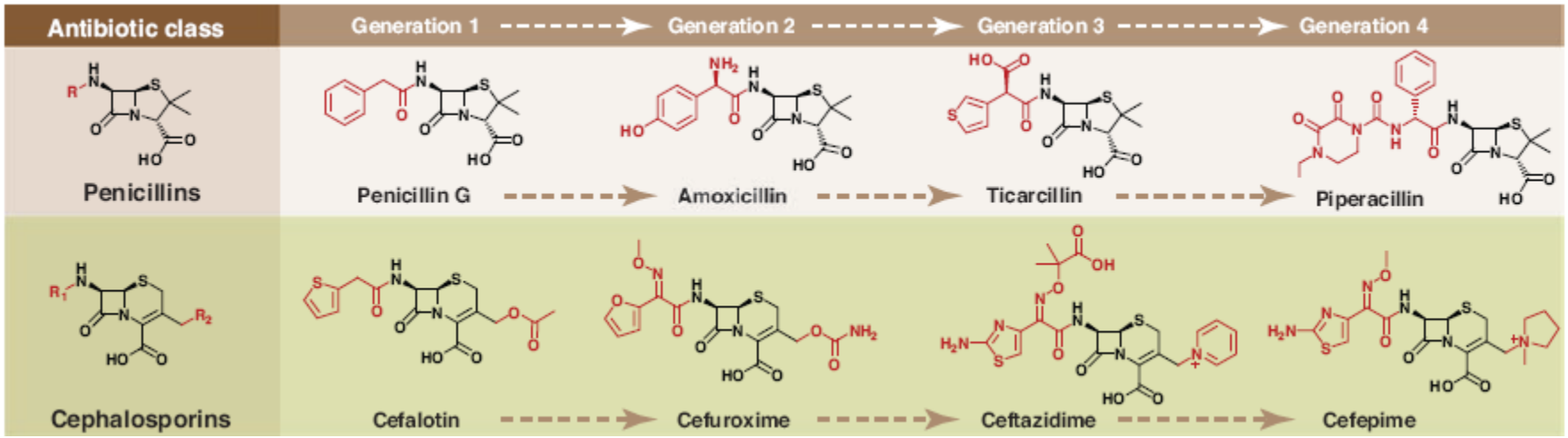
Penicillin Binds Irreversibly to its Target DD-Transpeptidase

Streptomyces DD-Transpeptidase
PDB ID 1pwg



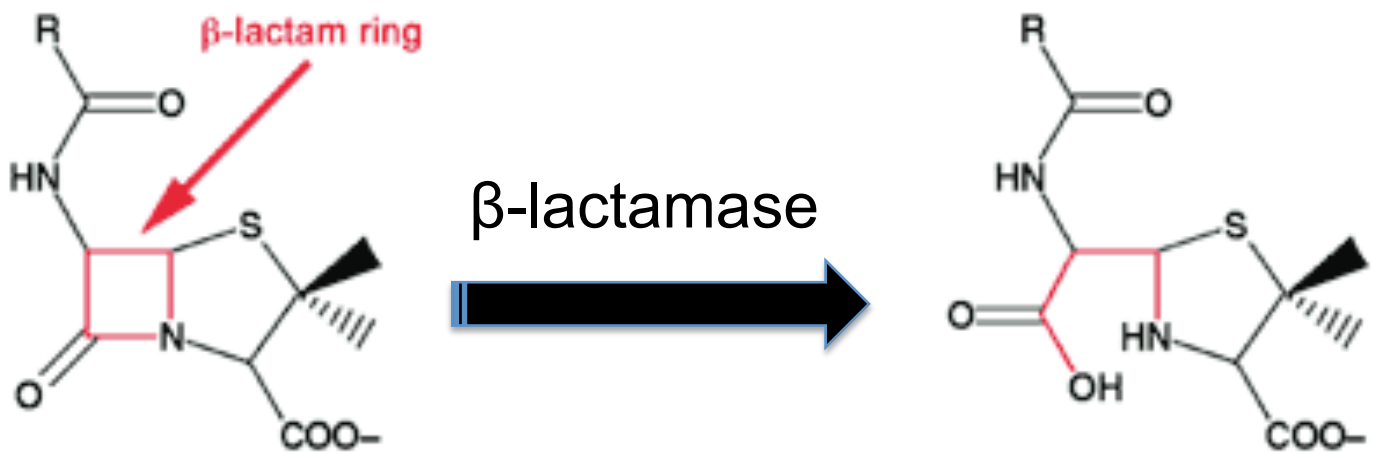
Antibiotic Arms Race I: New Drugs
More Penicillin Analogs then Cephalosporins

- Drug companies targeted DD-Transpeptidases with 4-6 bicyclic cephalosporin variations on the 4-5 bicyclic β-lactam scaffold common to Penicillins
- Cephalosporins are also D-Ala-D-Ala mimics



Discovery of Penicillin Resistance
β-Lactamase Enzymes

- Abraham and Chain discovered an enzyme that inactivates PCN
- Enzymes are proteins that act as catalysts enabling chemical reactions



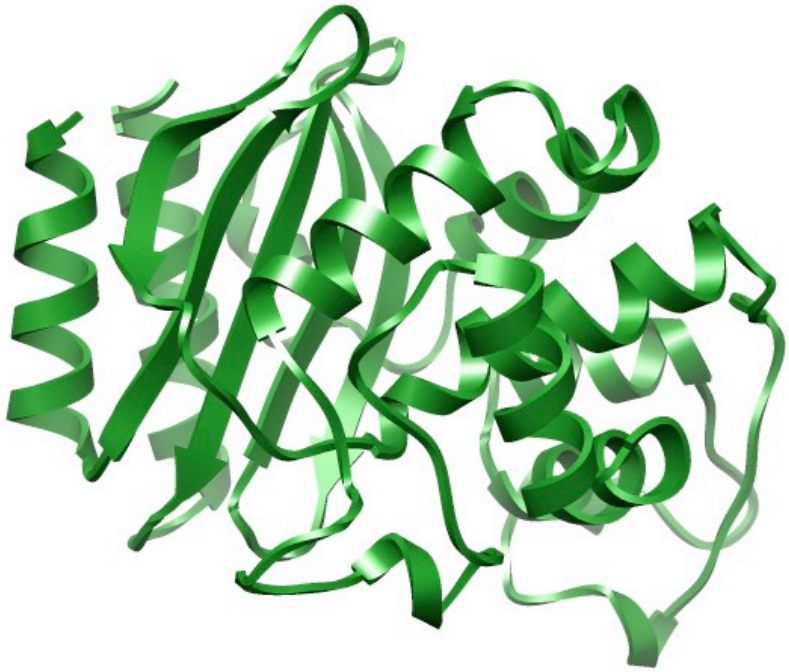
Penicillin
Antibiotic

Penicilloic acid
Inactive

Q: Where do β-Lactamases Come From?
A: Many Evolved from DD-Transpeptidases

Antibiotic Target →→→ Resistance Enzyme

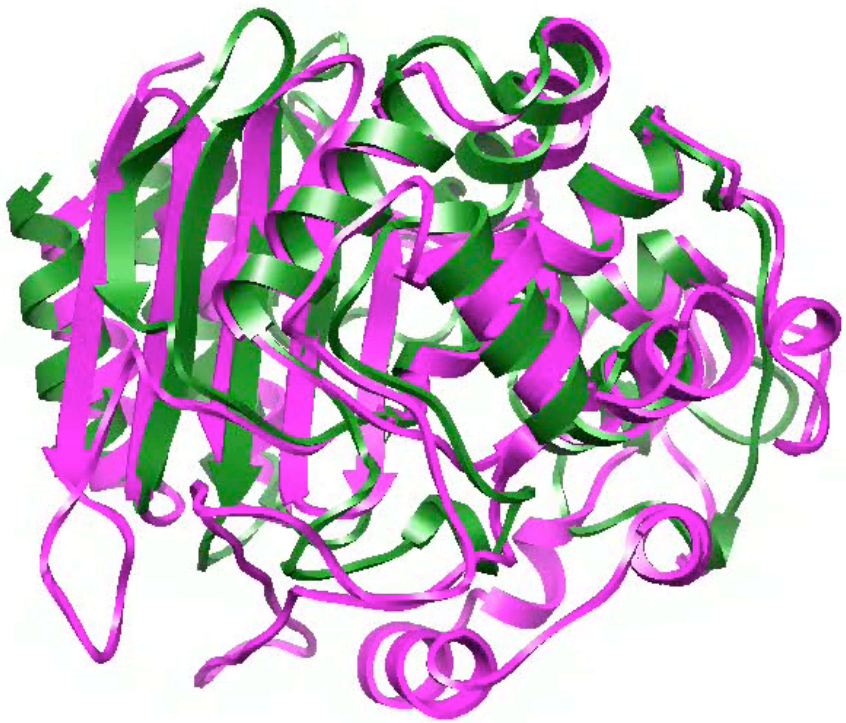
Streptomyces DD-Transpeptidase
PDB ID 1pwg

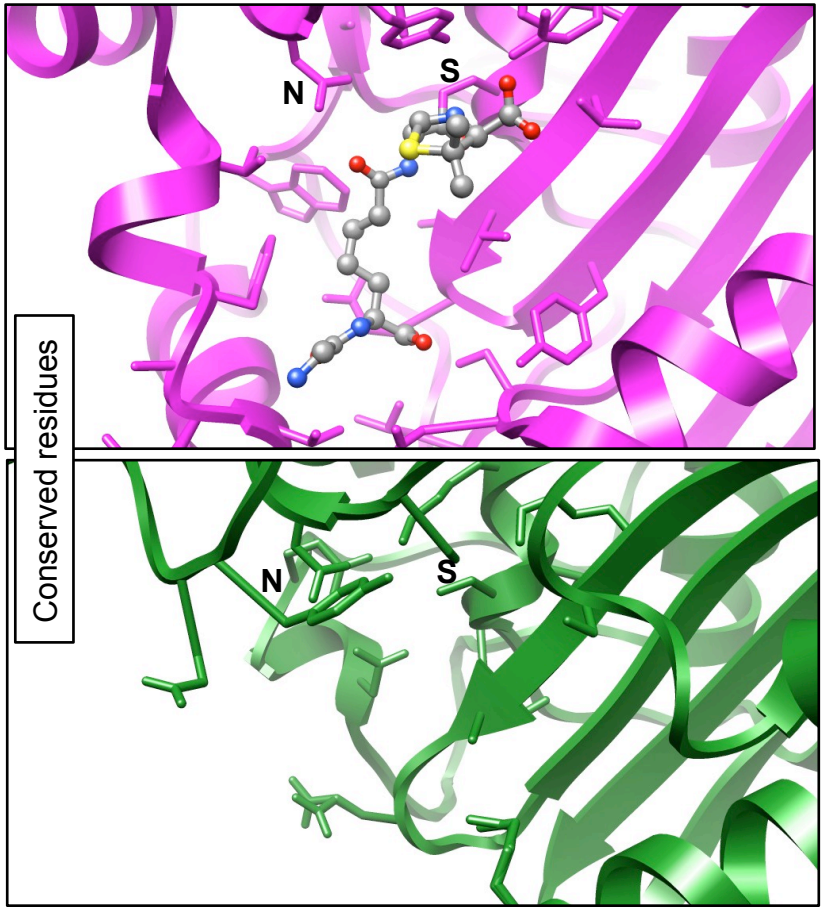


E.coli TEM-1 β-lactamase
PDB ID 1m40

β-Lactamases Evolved from DD-Transpeptidases (gene duplication then mutational changes)

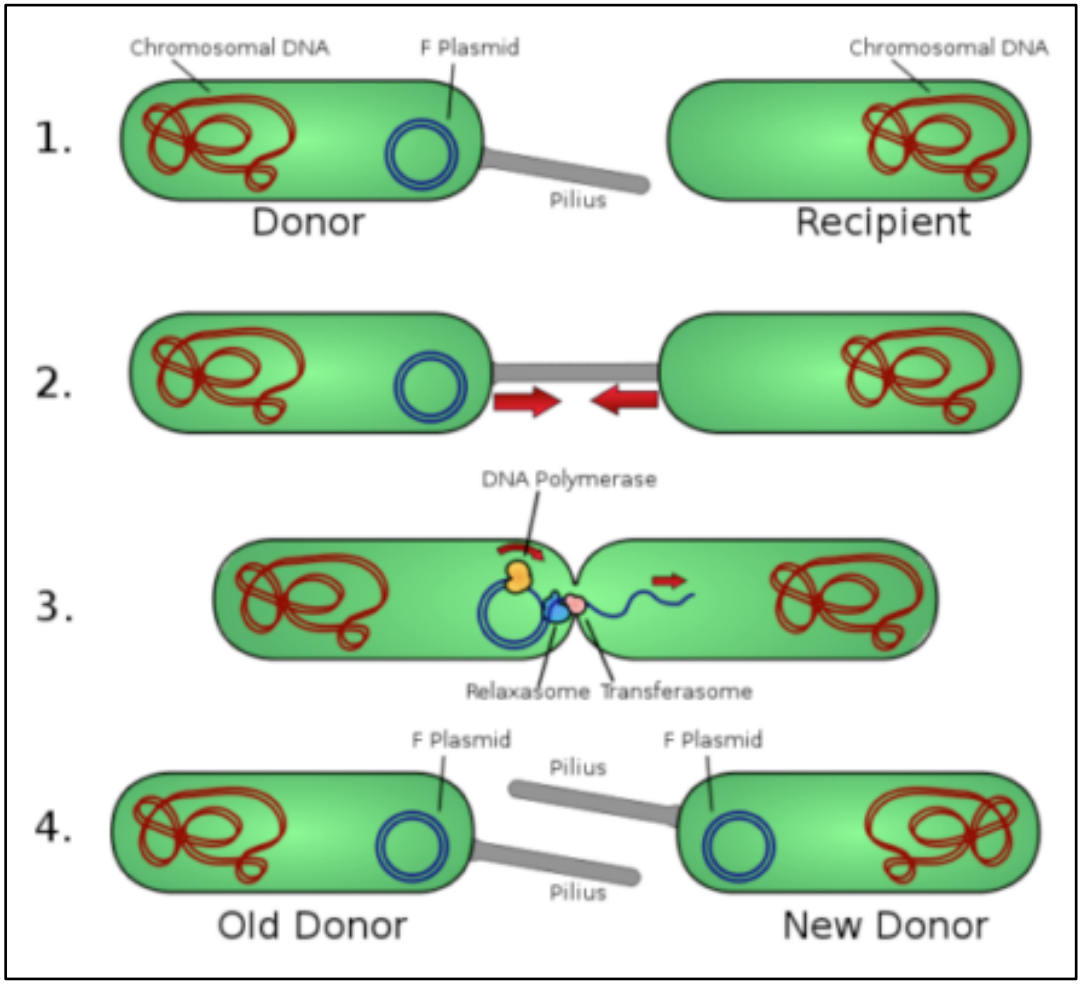
DD-Transpeptidase *versus* β-Lactamase





Transmission of Antibiotic Resistance

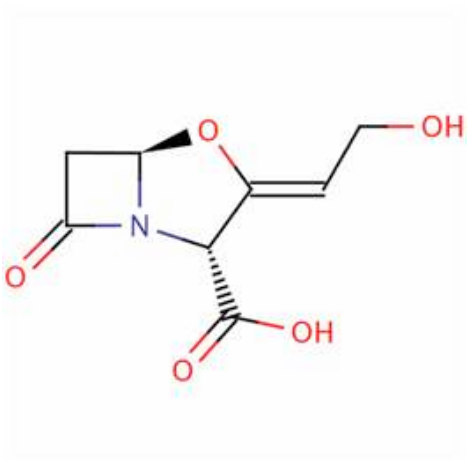
- Bacteria exchange DNA using Plasmids
- Resistance genes (β-lactamase genes, for example) can go global within a few years



Antibiotic Arms Race II: Combination Rx Penicillin + β-Lactamase Inhibitor

- Penicillin inactivation can be prevented by inhibiting the β-lactamase enzyme
- Clavulanic acid (another mimic of D-Ala-D-Ala) irreversibly blocks the action of β-lactamase

Clavulanic Acid



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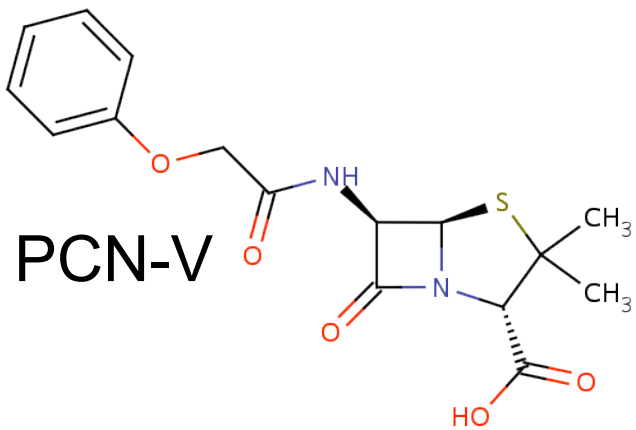
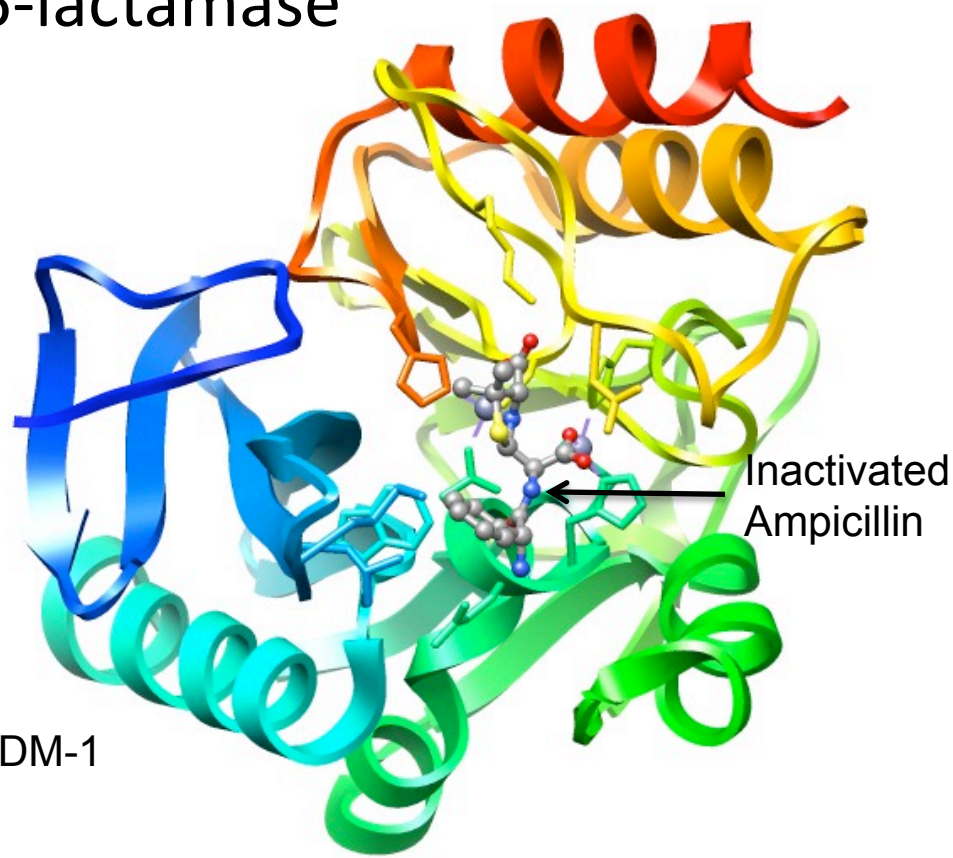
PCN-V



Antibiotic Arms Race III: Nature is Winning!!!!

- New Delhi "Superbugs" carry a gene encoding a new type of broad spectrum β-lactamase
- Metal-ion dependent enzyme (not like the DD-Transpeptidases)
- New Drug Target!

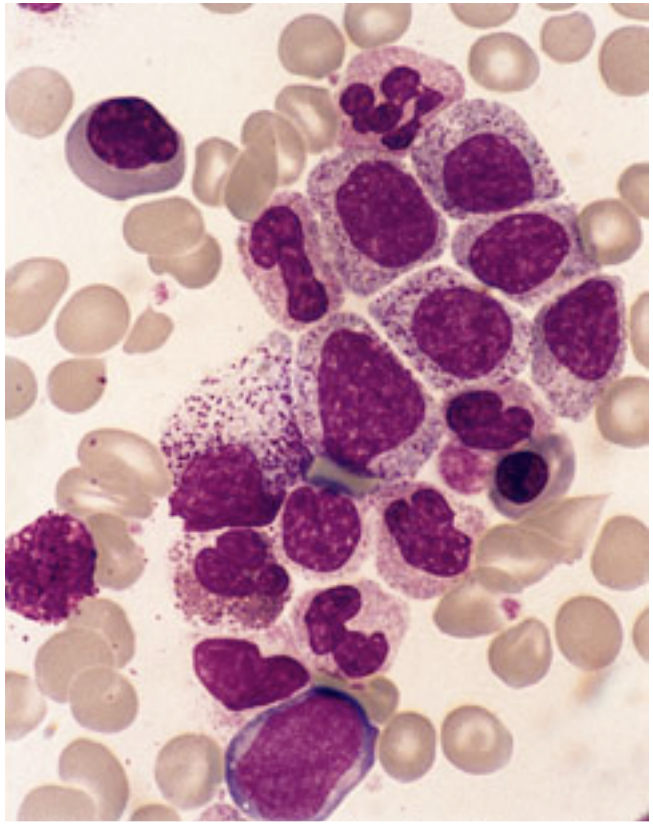


β-lactamase NDM-1
PDB ID 3q6x

Targeted Discovery of Gleevec

- White blood cell cancer
- Chronic Myeloid Leukemia (CML)
- Fatal before Gleevec
- 5yr Survival > 90%
- One Pill/Day

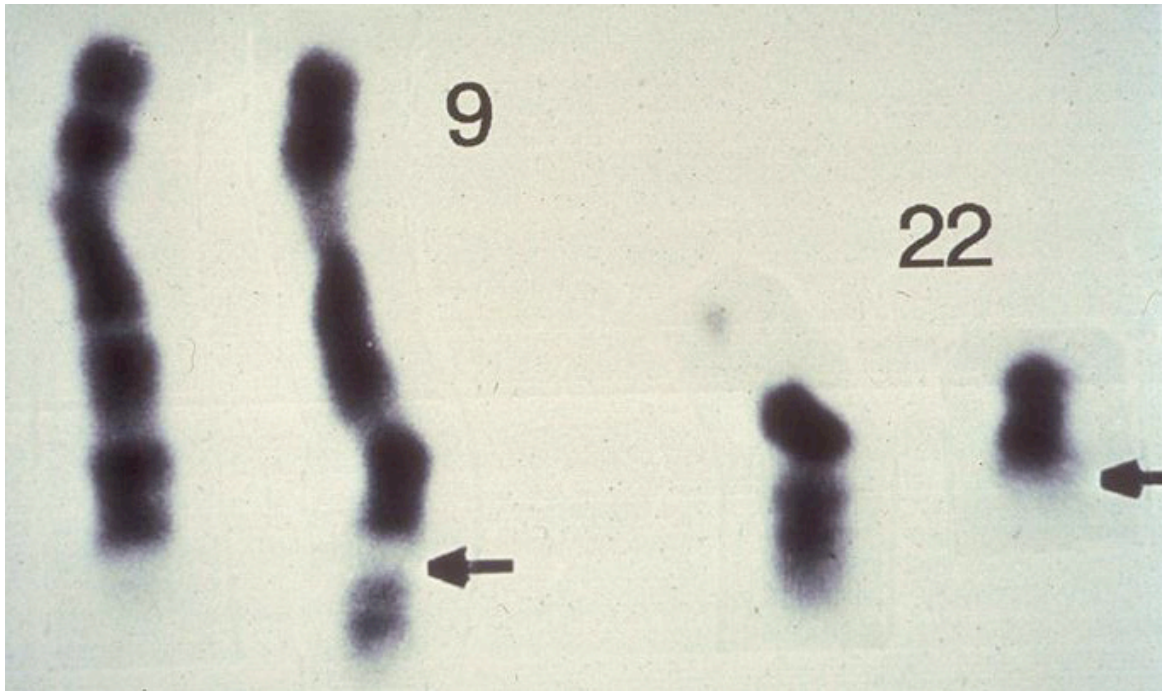
- Leukemic Blasts



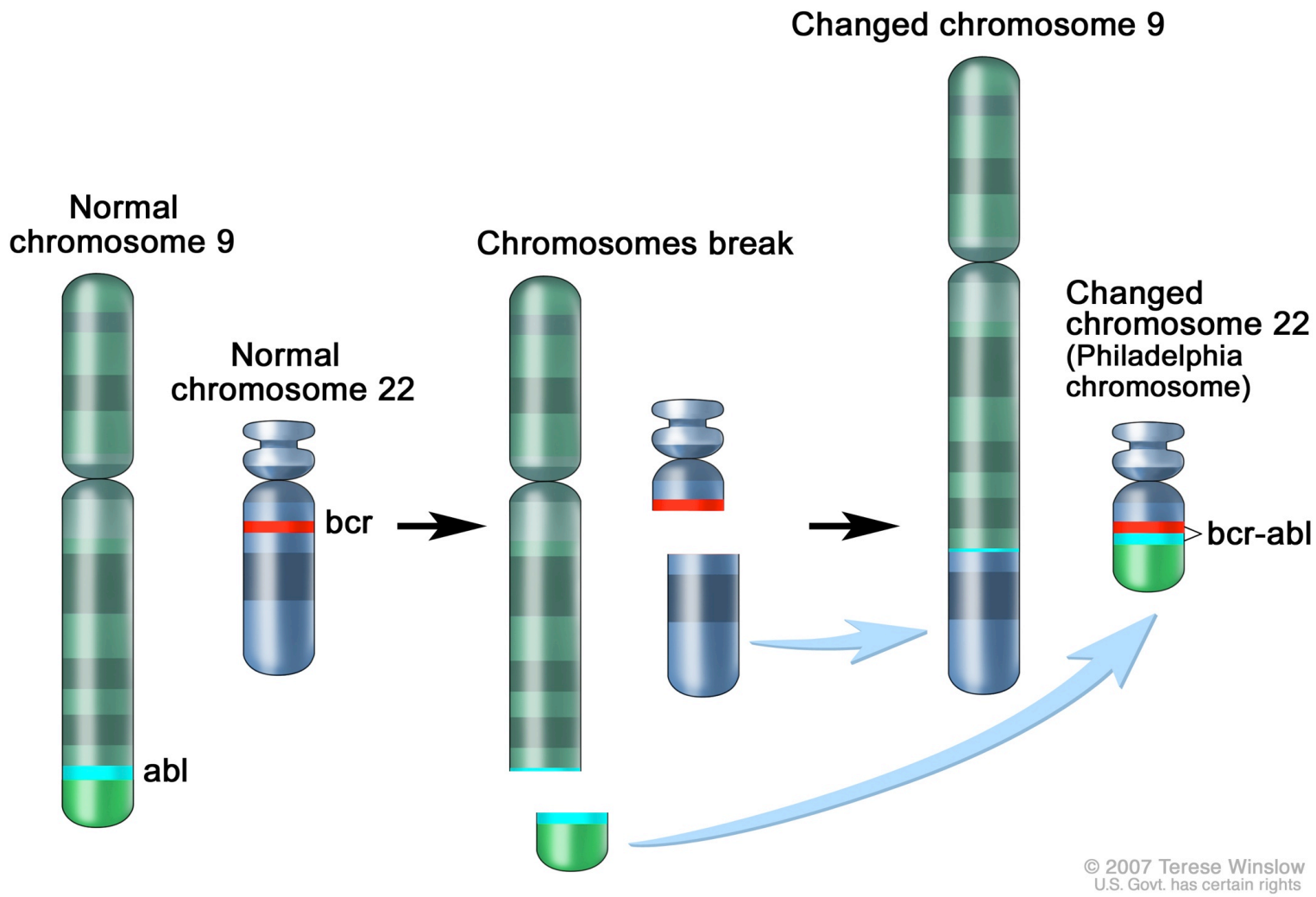
Chronic Myeloid Leukemia: One Hit Cancer

- Single DNA Abnormality
- Runaway Cell Growth
- "Cancer Genome"
- Targeted Therapy can "Correct" Abnormality

- All CML Cells have the Philadelphia Chromosome t(9:22)—Rowley (FCCC)

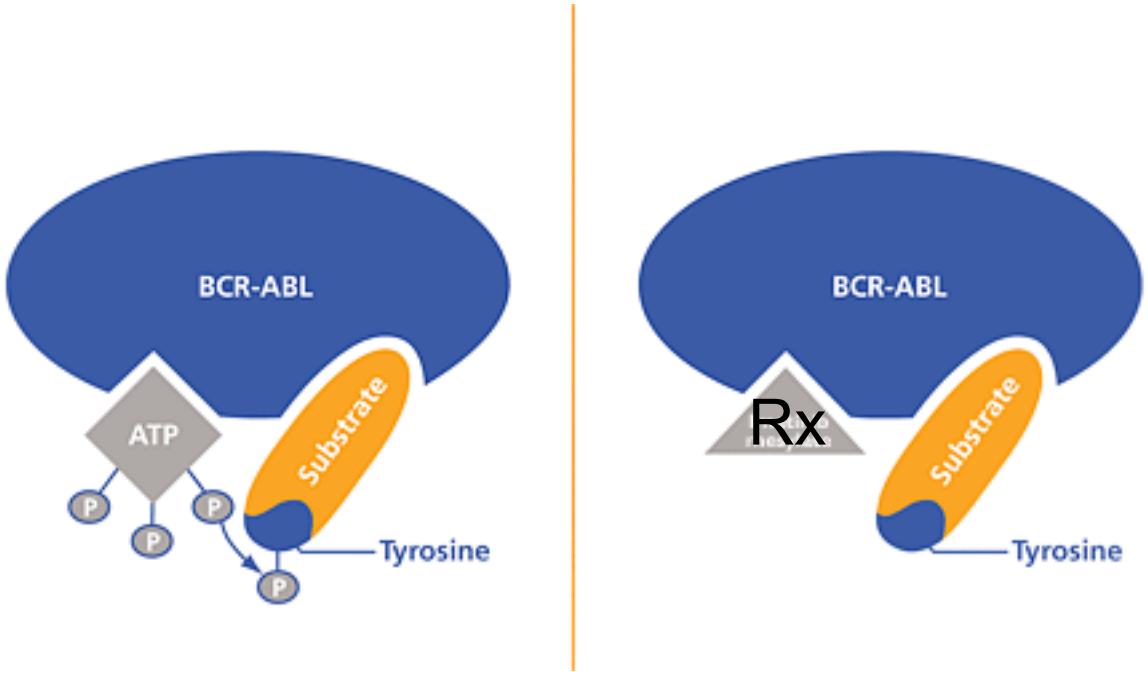


Chronic Myeloid Leukemia: What Goes Wrong I



Chronic Myeloid Leukemia: What Goes Wrong II

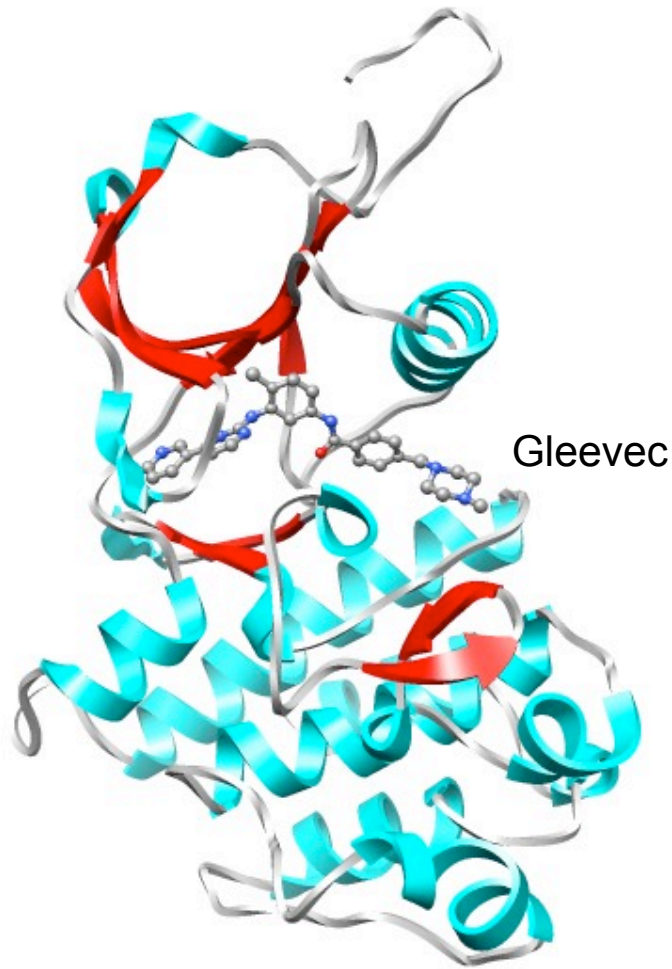
- t(9:22) translocation fuses BCR gene with ABL gene
- BCR-ABL gene→mRNA→BCR-ABL fusion protein
- BCR-ABL protein→↑↑↑Cell Growth→Cancer



BCR-ABL Enzyme: Target for CML Drug Discovery

- Gleevec (synthesized by Novartis) inhibits BCR-ABL by binding reversibly to the enzyme active site

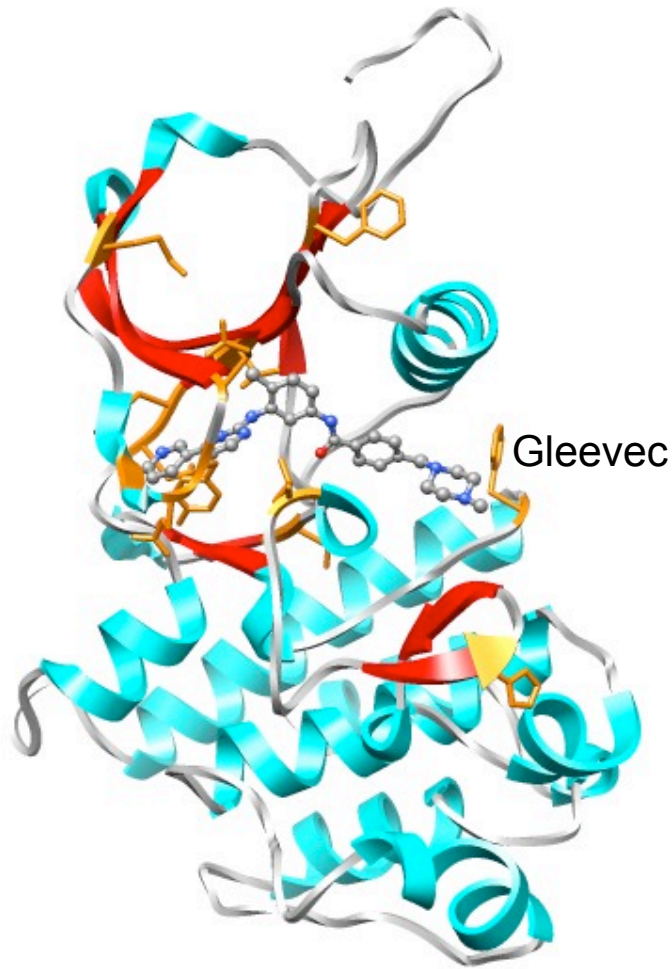
- Lydon (Novartis) and Druker (Oregon) collaborated to prove that CML could be treated with Gleevec and save lives



Gleevec Inhibition of BCR-ABL
PDB ID 1iep

Challenge of Gleevec Resistance

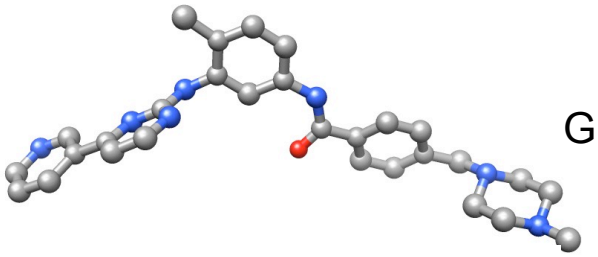
- Some patients are resistant to Gleevec
- Point mutations in BCR-ABL gene block binding of Gleevec
- We need a new drug!



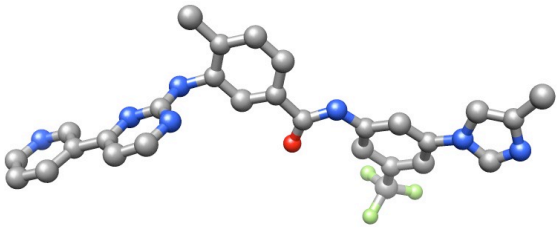
Mutations in BCR-ABL leading to Gleevec resistance PDB ID 1iep

Overcoming Gleevec Resistance: Medical Science is Winning the Race (for now)

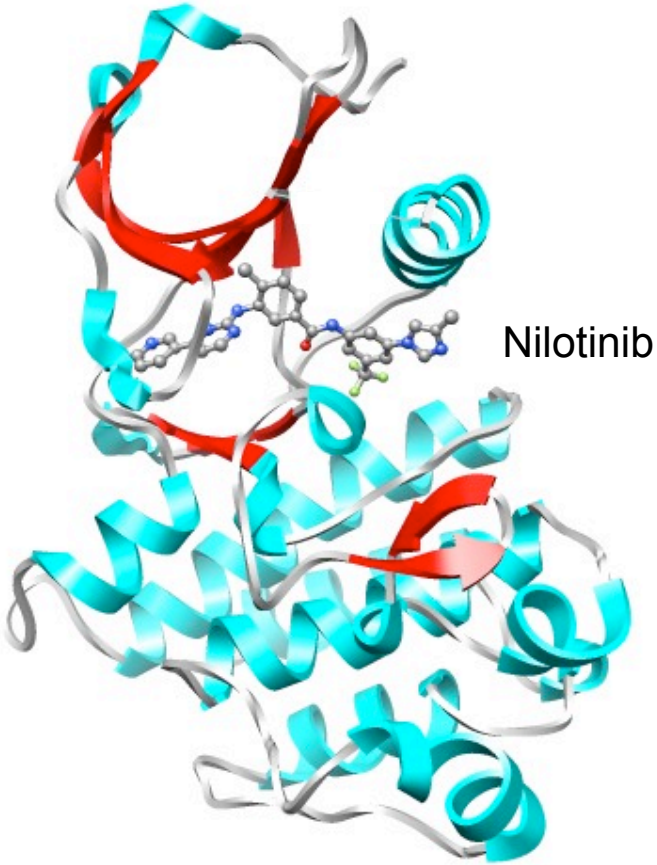
- Nilotinib (a Gleevec analog) inhibits BCR-ABL by binding reversibly to the enzyme active site
- Nilotinib works against most resistance mutations



Gleevec



Nilotinib



Inhibition of BCR-ABL with Nilotinib
PDB ID 3cs9

2012 Japan Prize
Discovery of Gleevec (Rowley, Druker, Lydon)

Learn More about Drugs from the PDB

- PDB-101 (http://www.pdb.org/pdb/101/structural_view_of_biology.do)
- How do Drugs Work (downloadable poster)
- Molecule of the Month
  - Drug Action
  - Molecular Basis of Disease
  - Antibodies

Final Words of Advice

- "DNA makes RNA makes Protein"
- In Biology, "Function follows Form"
- Career Advice: Today's Biomedical Research combines Biology and Medicine with Chemistry, Physics, Mathematics, and Computer Science