Enzymes as Drug Targets

B.Sc. Pharmacology & Translational Medical Science, yr 2

Marc-Emmanuel Dumas, Ph.D.
Biomolecular Medicine, Dept Surgery and Cancer
Sir Alexander Fleming Building, room 360
South Kensington Campus
m.dumas@imperial.ac.uk

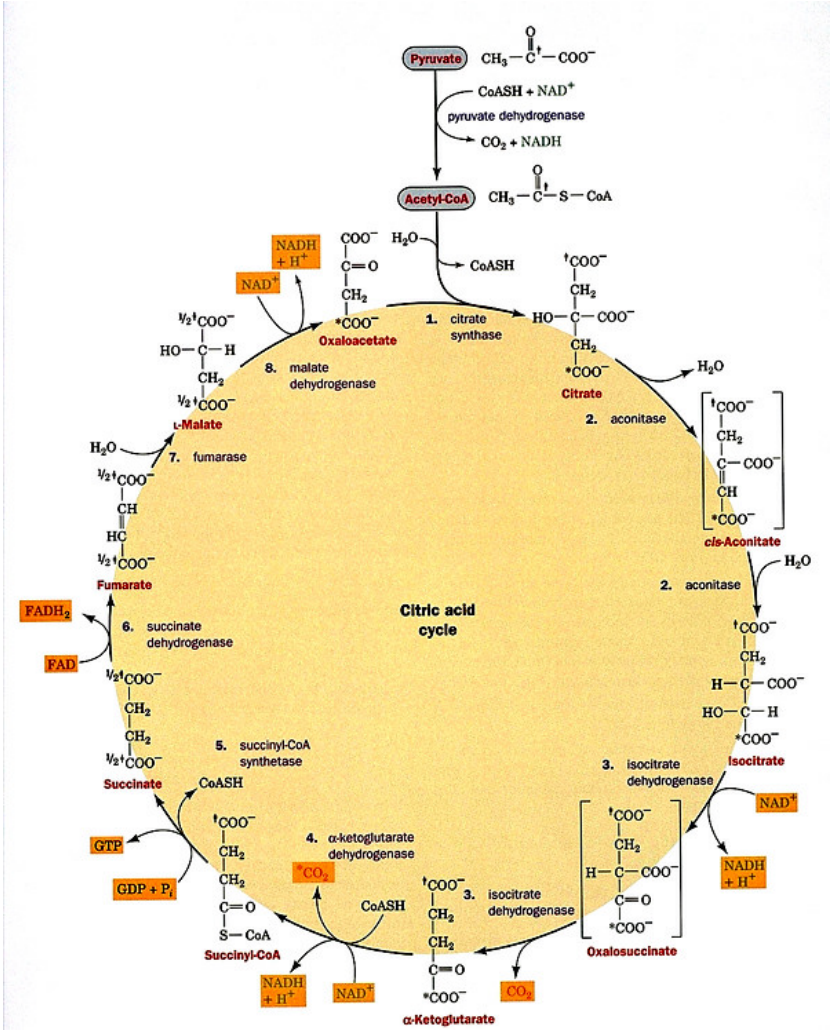
Learning objectives

- Enzymes and their biological function
- Active sites and catalysis mechanisms
- Different types of Inhibition
  - Competitive inhibition
  - Irreversible inhibition
  - Allosteric (reversible) inhibition
- Examples

Enzymes and their biological function

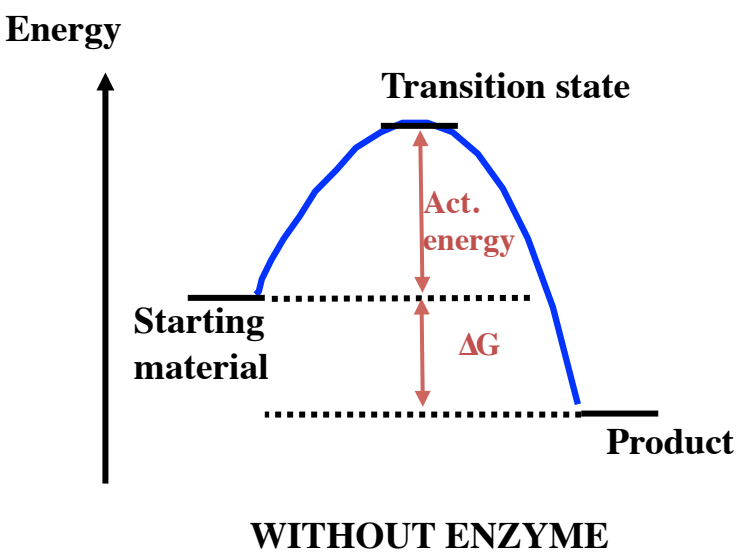
What is an enzyme?

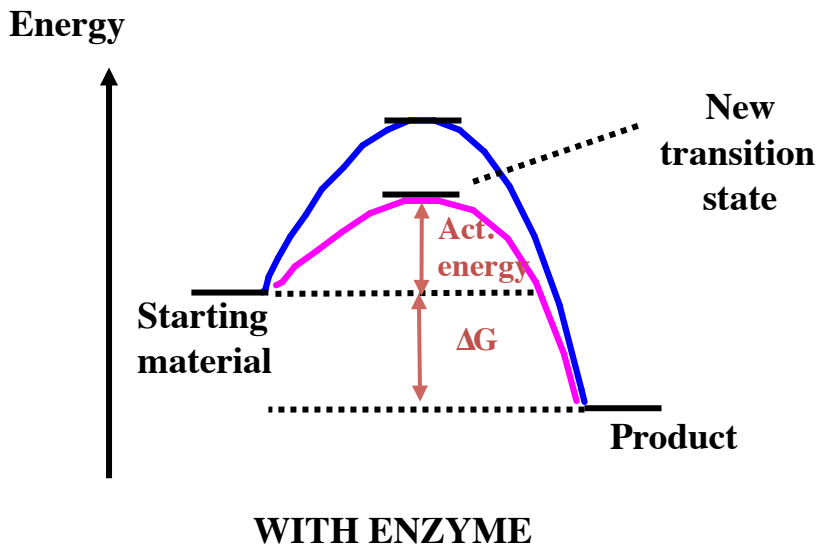
- Globular proteins acting as the body's catalysts
- Nomenclature:
  - Root + ase
- Classification
  - Oxidoreductases
  - Transferases
  - Hydrolases
  - Lyases
  - Isomerases
  - Ligases/synthetases



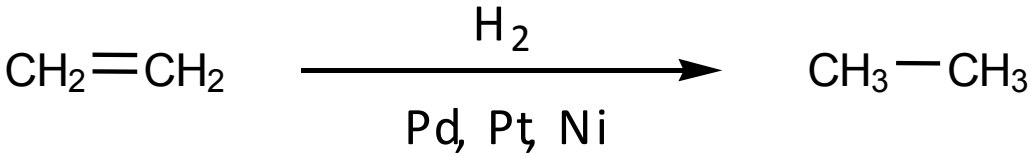
What is a catalyst?

- Speed up time for reaction to reach equilibrium
- Lowers activation energy

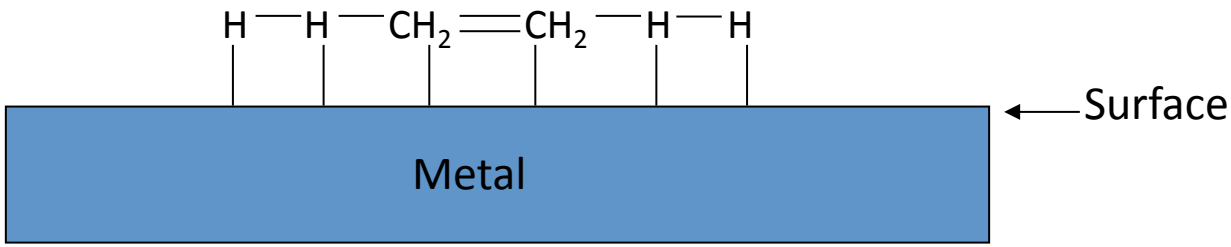




Methods of catalysis



- **Provide a reaction surface/suitable environment**
- **Bring reactants together**
- **Position reactants correctly for reaction**
- **Weaken bonds in the reactants**



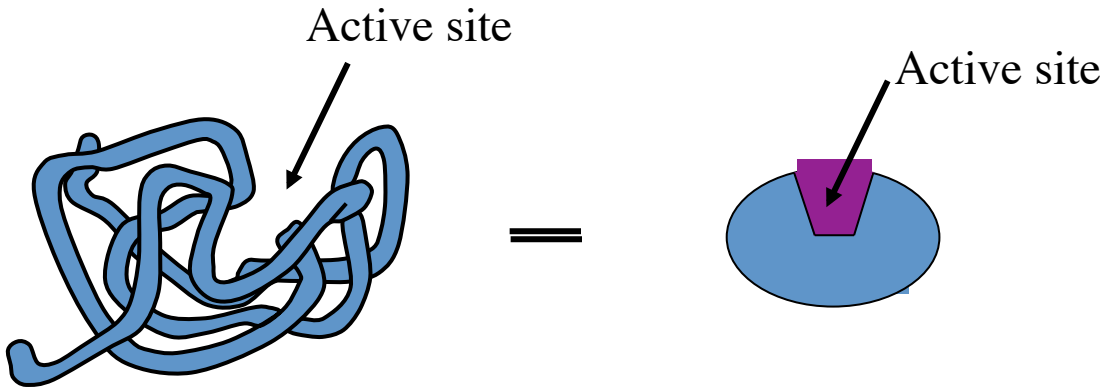
Enzymes as catalysts

- **Provide a reaction surface (the active site)**
- **Provide a suitable environment (hydrophobic)**
- **Bring reactants together**
- **Position reactants correctly for reaction**
- **Weaken bonds in the reactants**
- **Provide acid / base catalysis**
- **Provide nucleophiles**

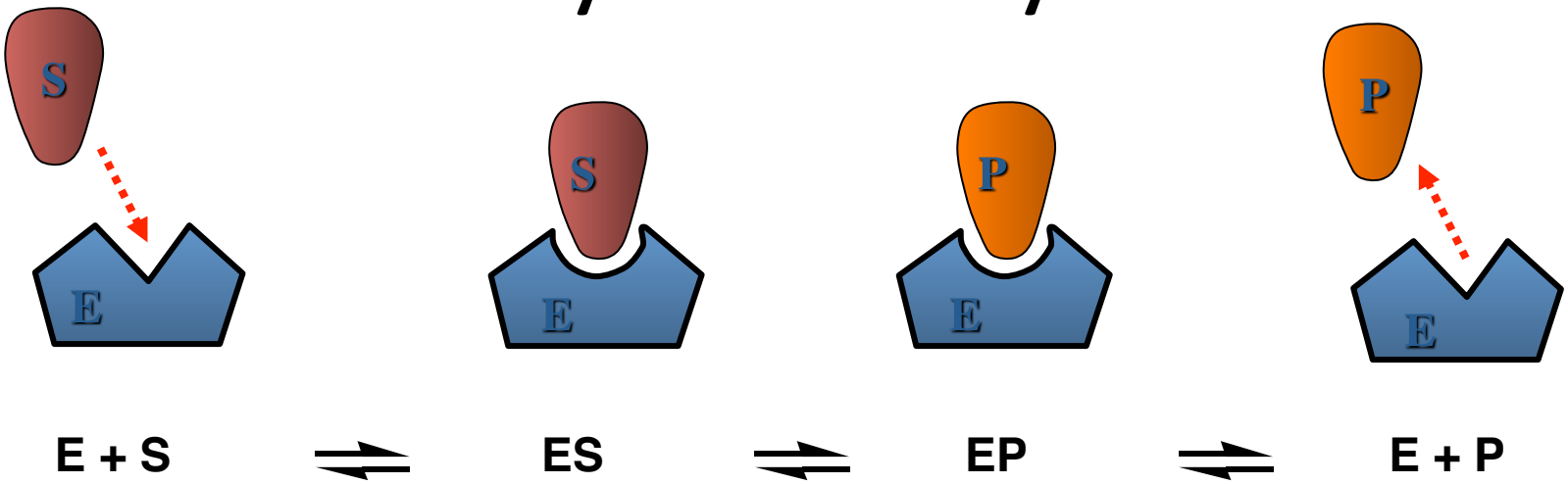
Active sites and catalysis mechanisms

The active site

- Hydrophobic hollow or groove on the enzyme surface
- Accepts reactants (substrates and coenzymes)
- Contains amino acids which
  - bind reactants (substrates and coenzymes)
  - catalyze the reaction

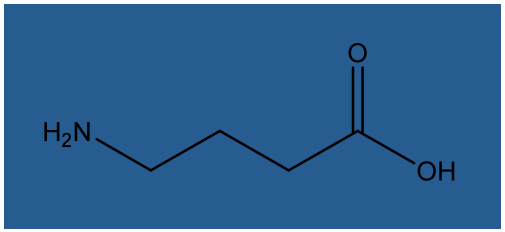


Enzyme catalysis

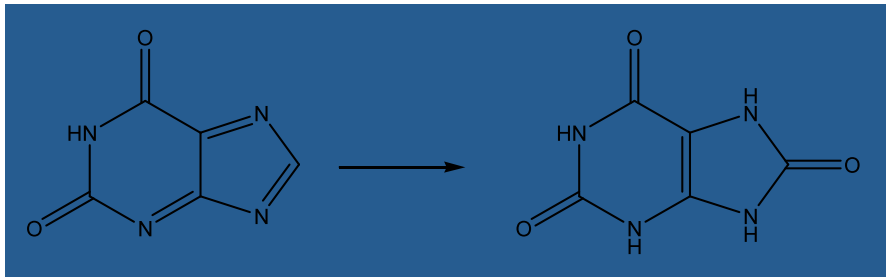


- **Binding interactions:**
  - **Ionic, H-bonding, van der Waals**
  - **strong enough to hold the substrate sufficiently long for the reaction to occur**
  - **weak enough to allow the product to depart**
- **Drug design:**
  - **designing molecules with stronger binding interactions results in enzyme inhibitors which block the active site**

Examples



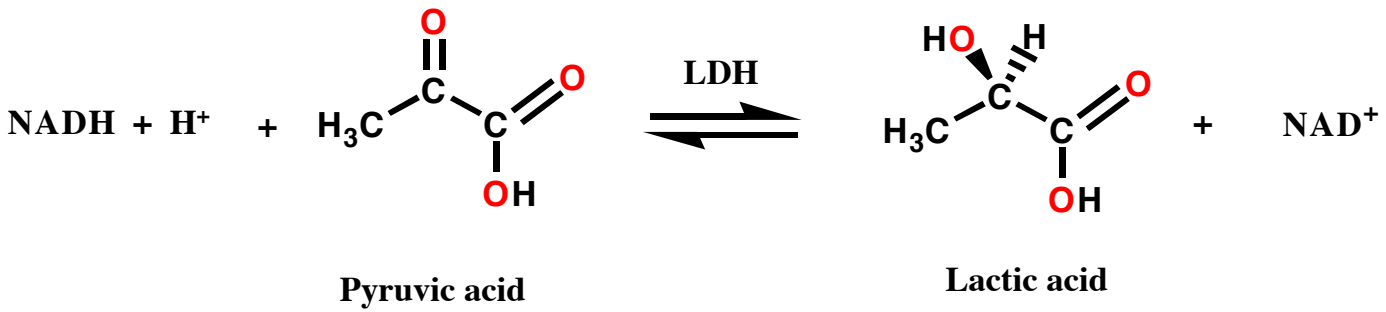
- Increase [S]
  - Decreased levels of GABA cause seizures
  - GABA aminotransferase degrades GABA
  - Inhibition of enzyme raise GABA levels



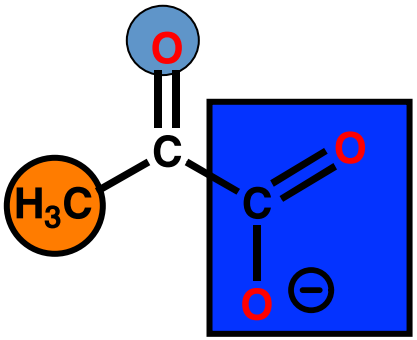
- Decrease [P]
  - Xanthine converts to uric acid with xanthine oxidase
  - Excess uric acid leads to gout
  - Inhibition of enzyme lowers production of uric acid

An example reaction:

- Reduction of pyruvate to lactate
- Homolactic fermentation
- LDH = Lactate dehydrogenase (enzyme)
- NADH = reducing agent & coenzyme
- Pyruvic acid = substrate

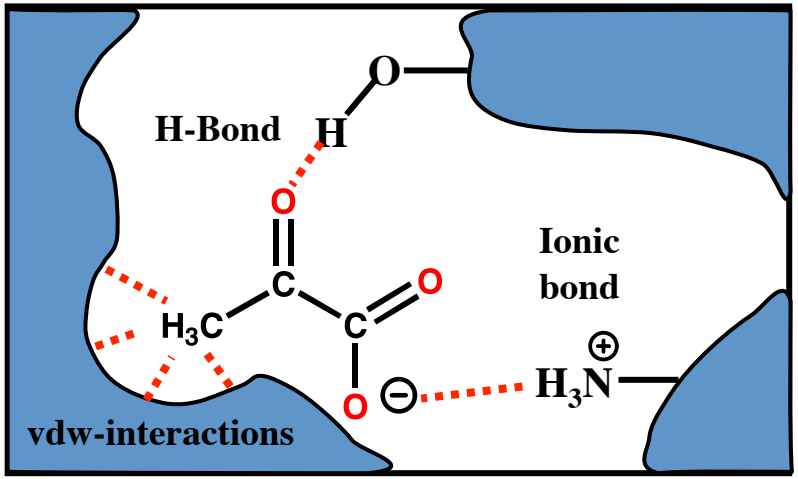


Binding of pyruvic acid in LDH



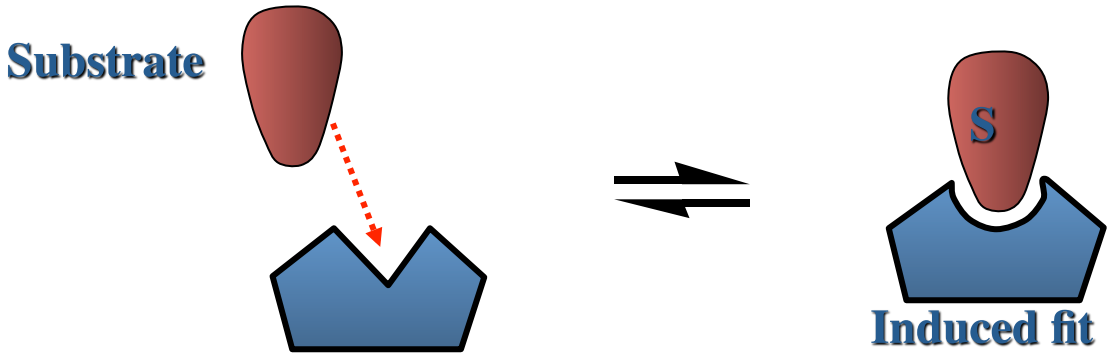
Possible interactions

- H-Bond
- van der Waals
- Ionic



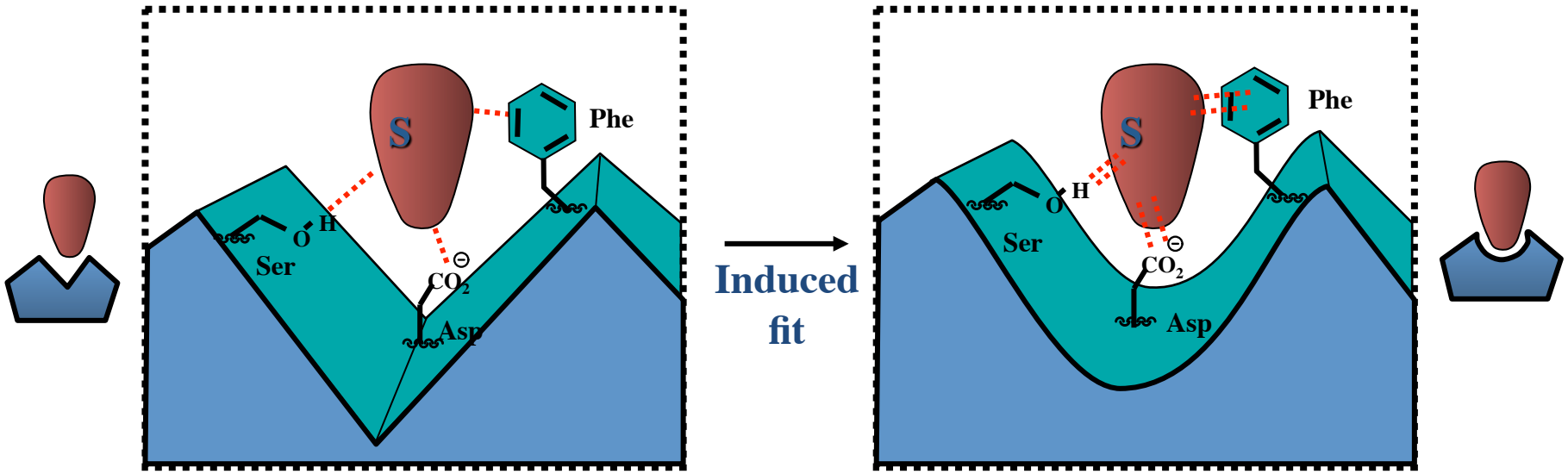
Substrate binding: induced fit

- Active site is nearly the correct shape for the substrate
- Binding alters the shape of the enzyme (induced fit)
- Binding will strain bonds in the substrate



Induced fit

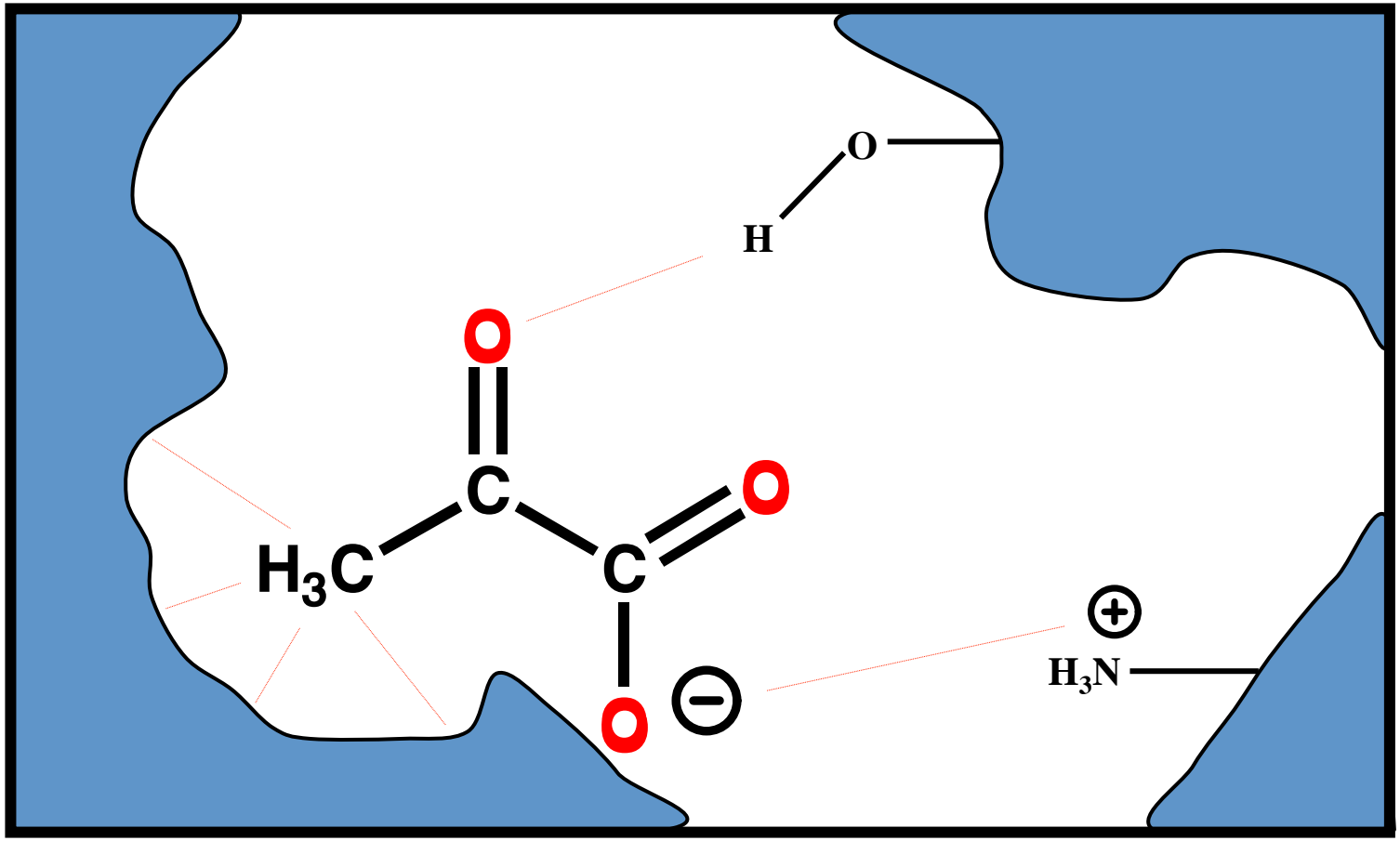
- Active site alters shape to maximize intermolecular attractions



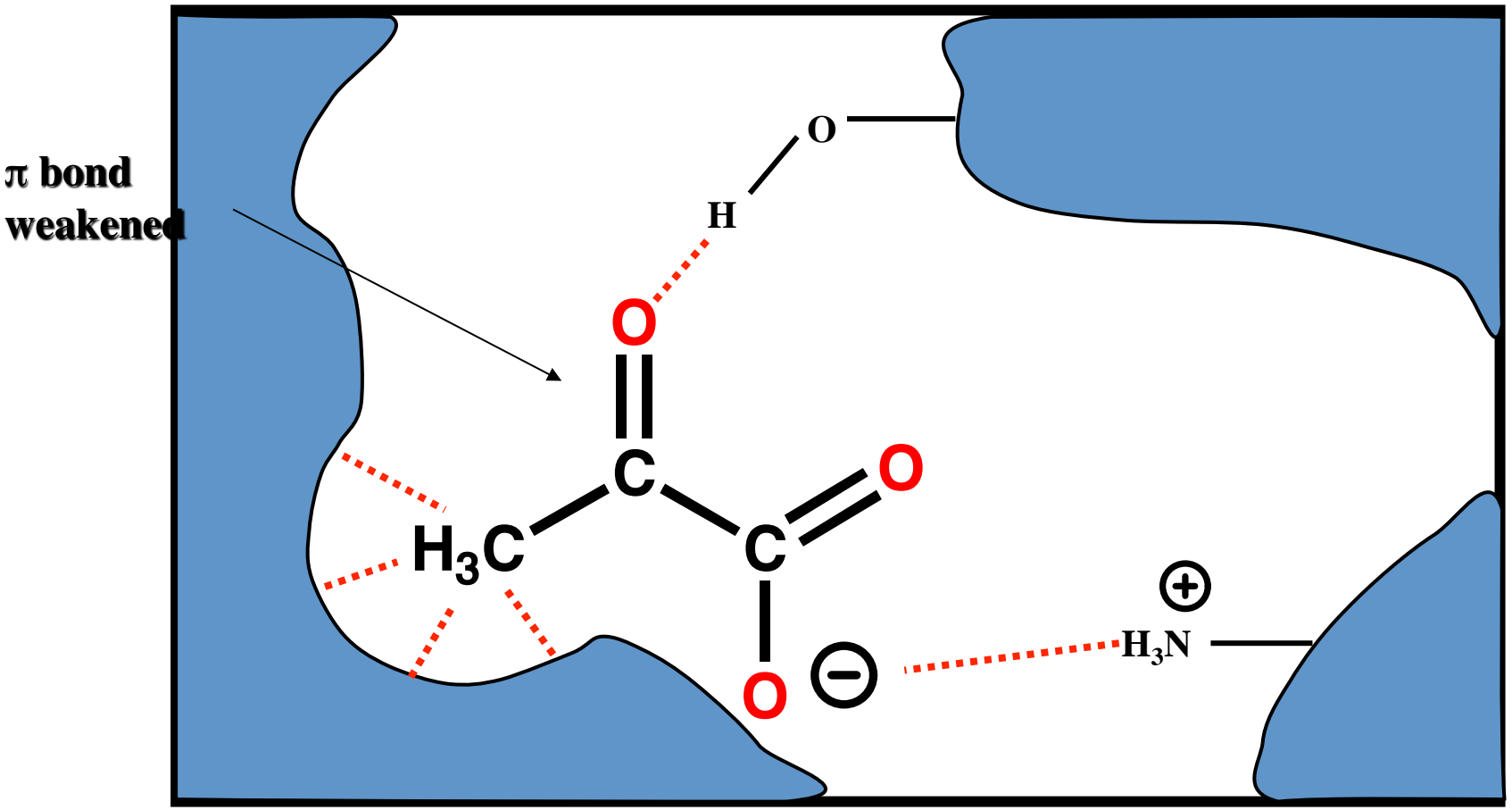
Intermolecular bonds not optimum length for maximum bonding

Intermolecular bond lengths optimized
Susceptible bonds in substrate strained
Susceptible bonds in substrate more easily broken

Binding of pyruvic acid in LDH

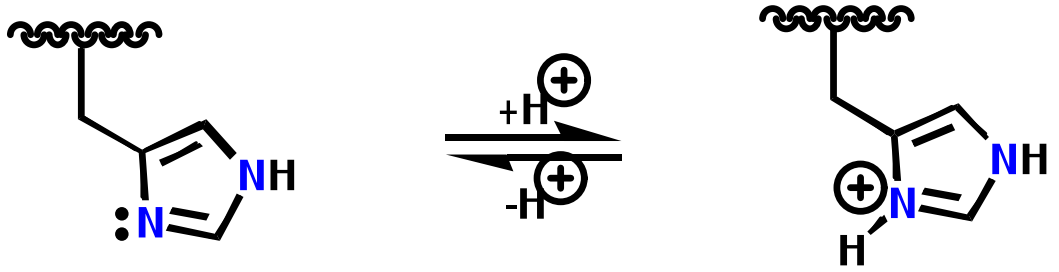


Binding of pyruvic acid in LDH



Catalysis Mechanisms

- Acid/base catalysis

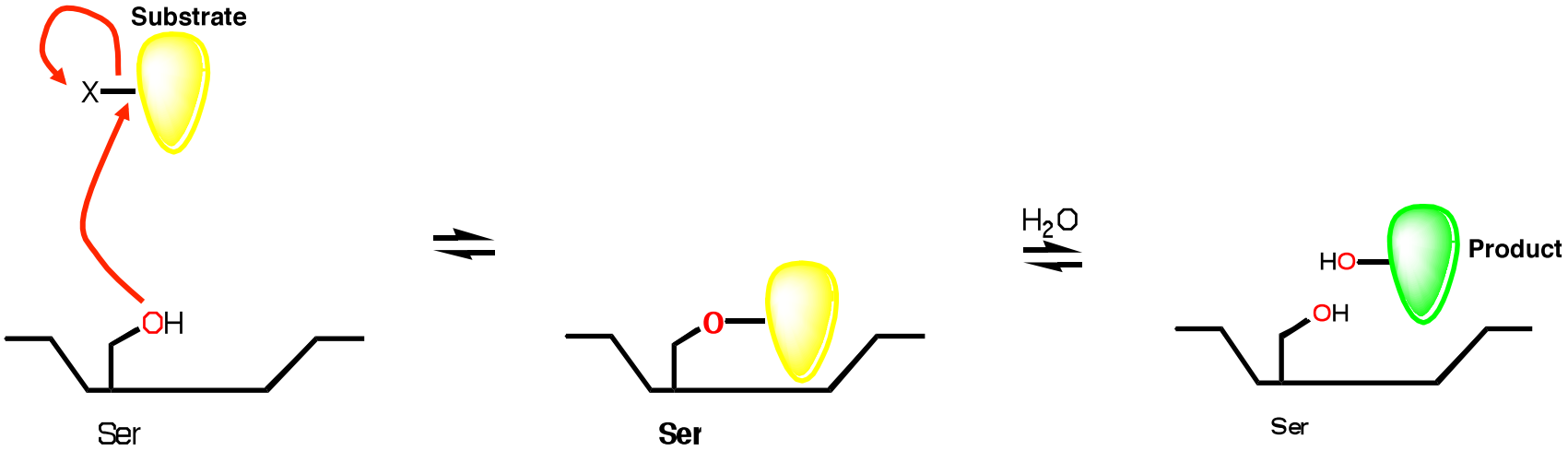


Non-ionized
Acts as a basic catalyst
(proton 'sink')

Ionized
Acts as an acid catalyst
(proton source)

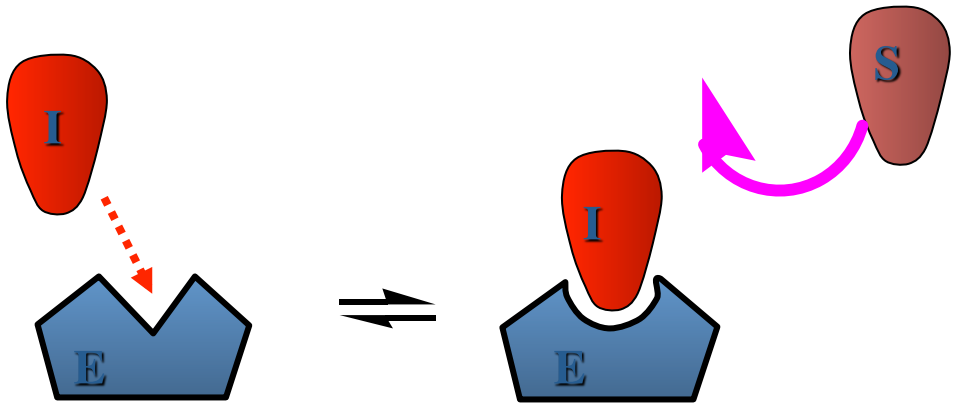
- Covalent catalysis: Nucleophilic residues

Serine as a nucleophile

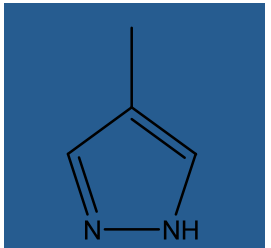


Different types of inhibition

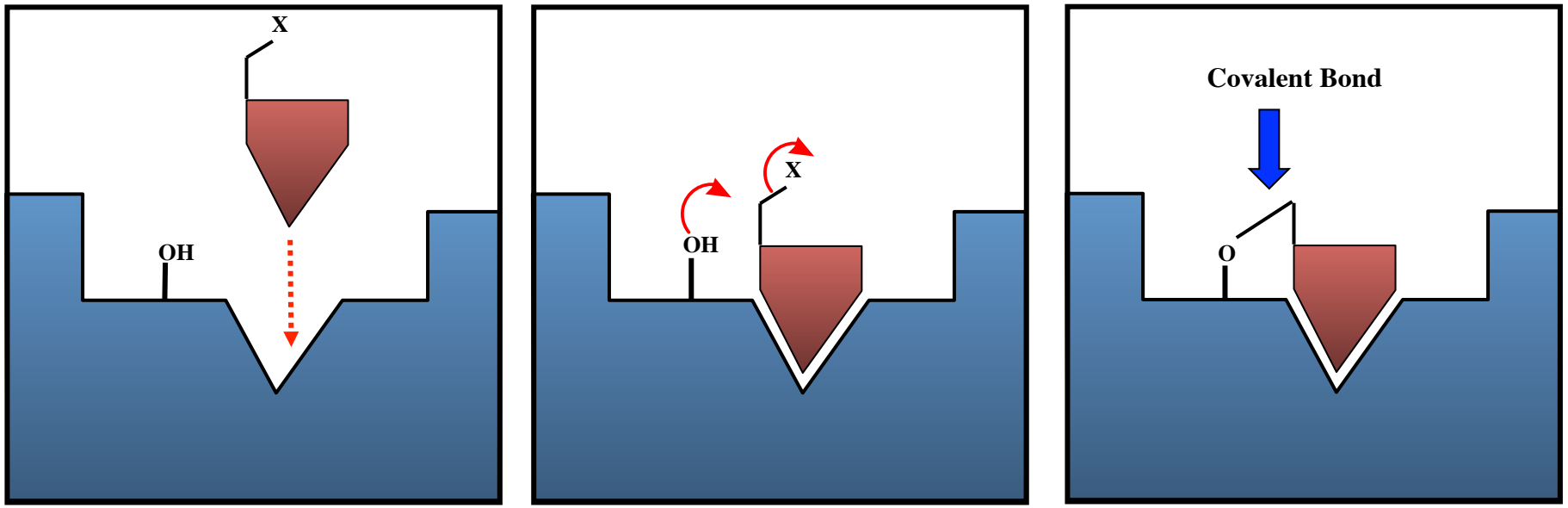
Competitive (reversible) inhibitors



- Inhibitor binds reversibly to the active site
- No reaction takes place on the inhibitor
- Inhibition depends on the strength of inhibitor binding and inhibitor concentration
- Substrate is blocked from the active site
- Increasing substrate concentration reverses inhibition
- Inhibitor likely to be similar in structure to the substrate
- Examples:
  - Ethylene glycol or methanol poisoning
  - Sulfonamide antibacterial agents (sulfa drugs)



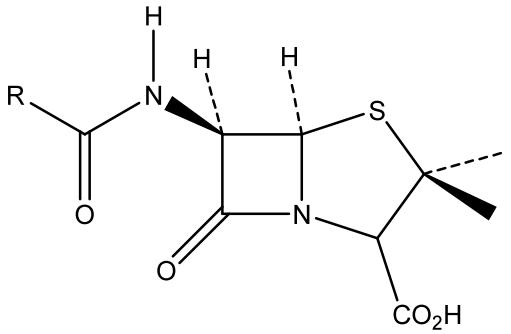
Irreversible inhibition



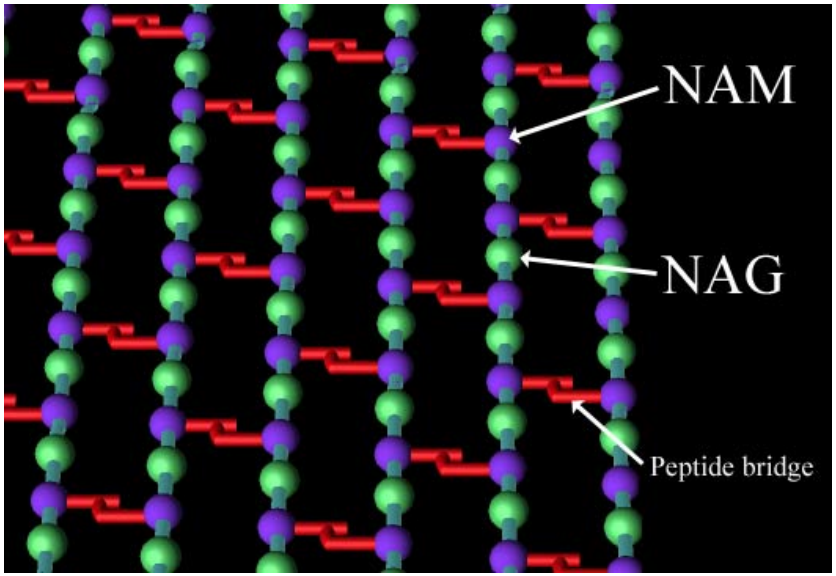
Irreversible inhibition

- Inhibitor binds irreversibly to the active site
- Covalent bond formed between the drug and the enzyme
- Substrate is blocked from the active site
- Increasing substrate concentration does not reverse inhibition
- Inhibitor likely to be similar in structure to the substrate
- Examples:
  - Aspirin
  - Penicillins

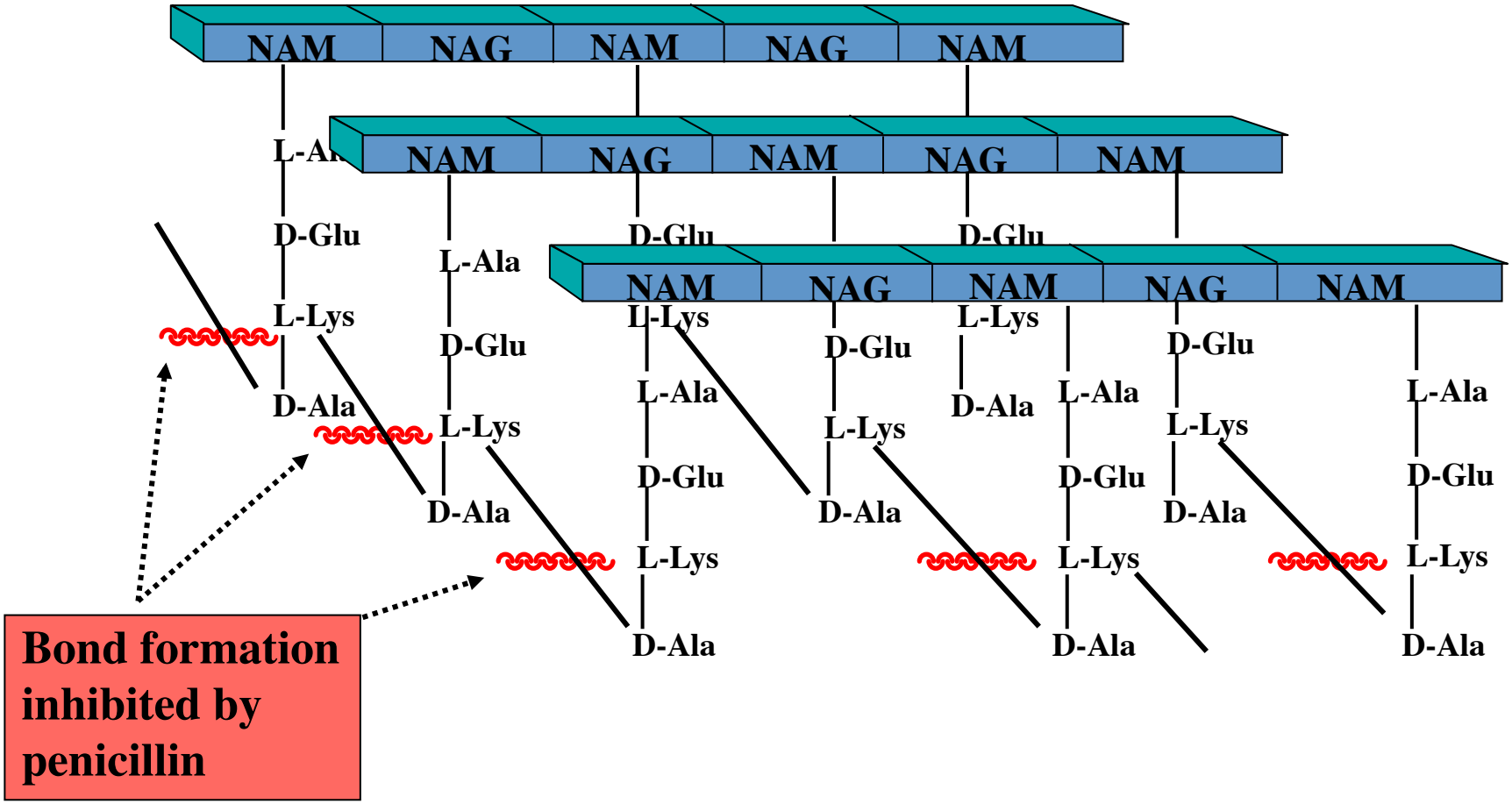
Penicillin



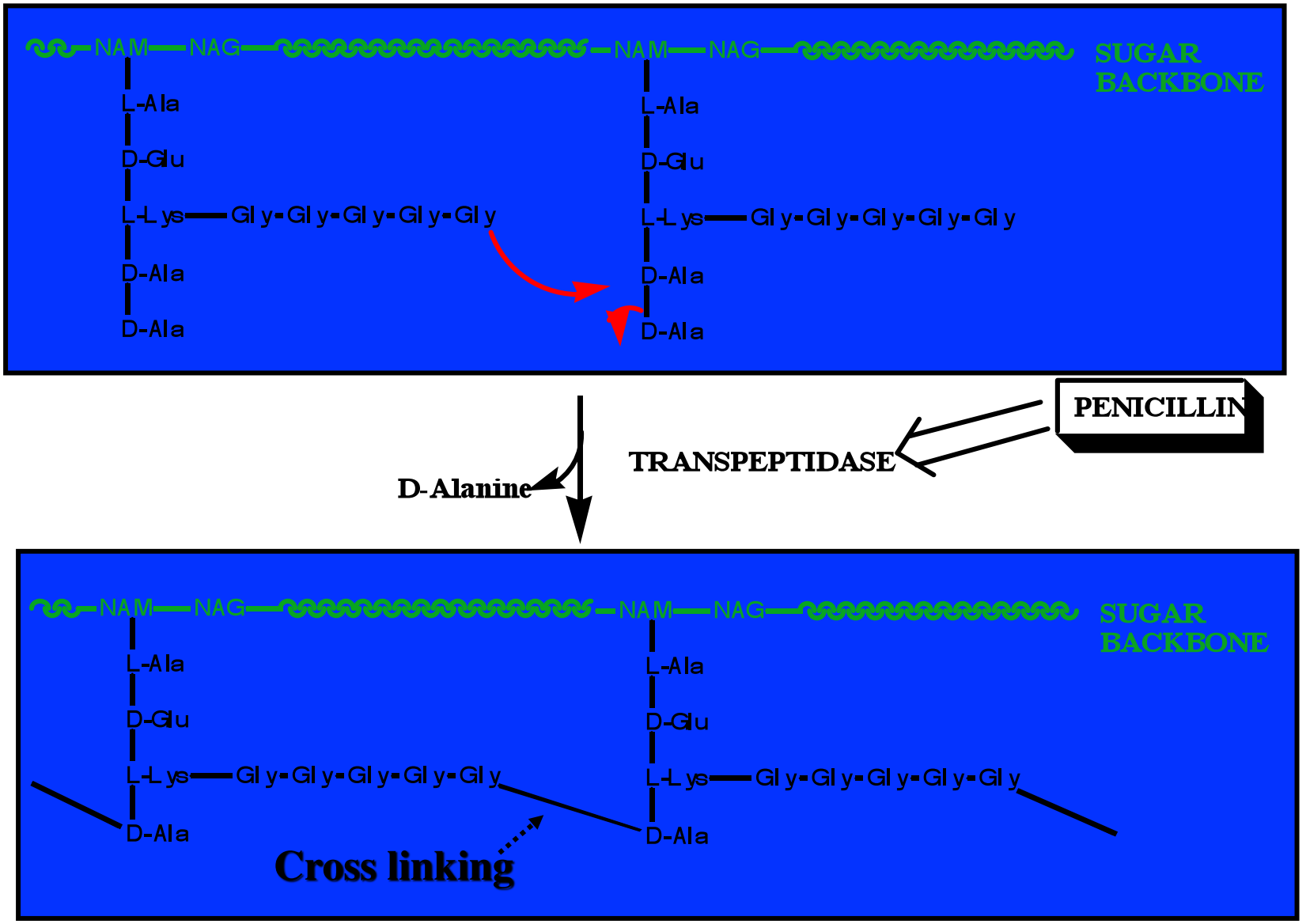
- Penicillin: antibiotic binding bacterial transpeptidase (discovered by Sir Alexander Fleming)
- Peptidoglycan cell wall: overlapping lattice of 2 sugars - N-acetyl glucosamine (NAG) and N-acetyl muramic acid (NAM) - that are crosslinked by peptide bridges



Mechanism of action of penicillin

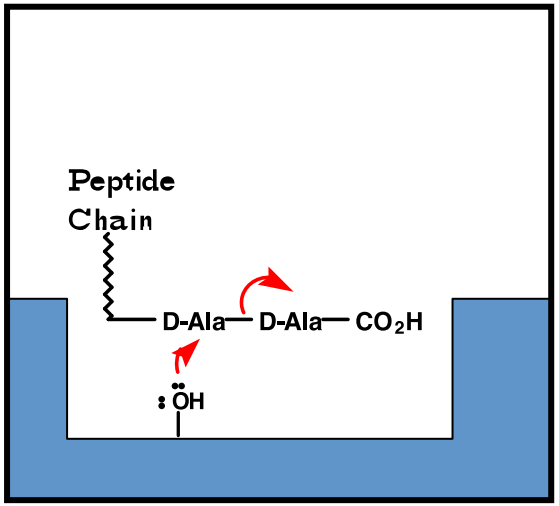


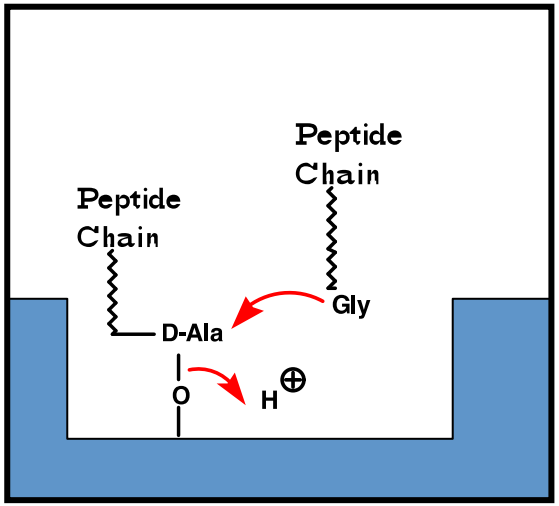
Bacterial cell wall synthesis

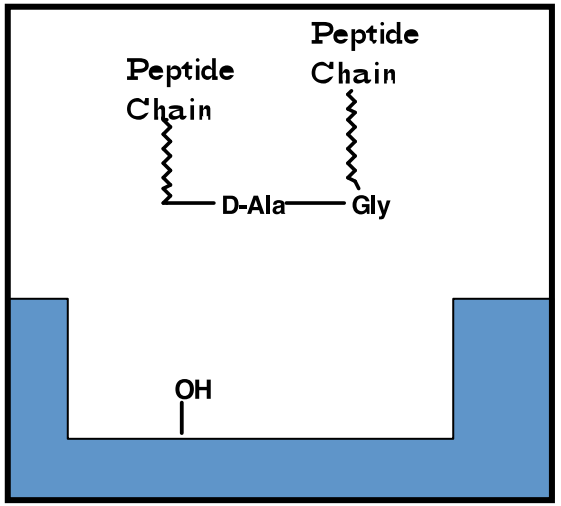


Bacterial cell wall synthesis

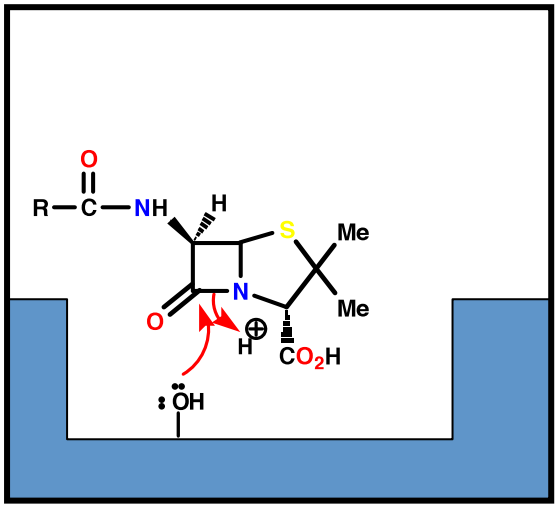
Normal Mechanism:

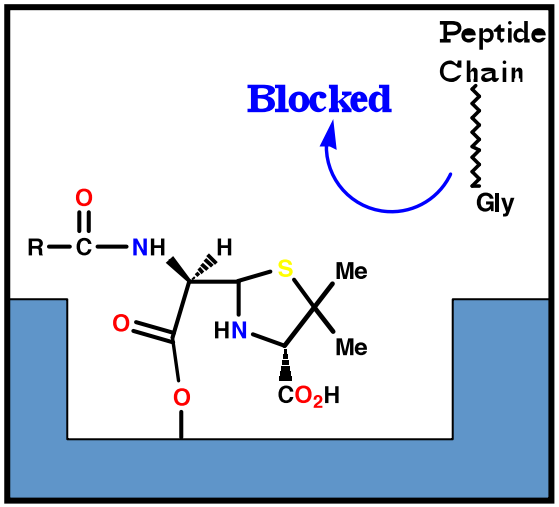


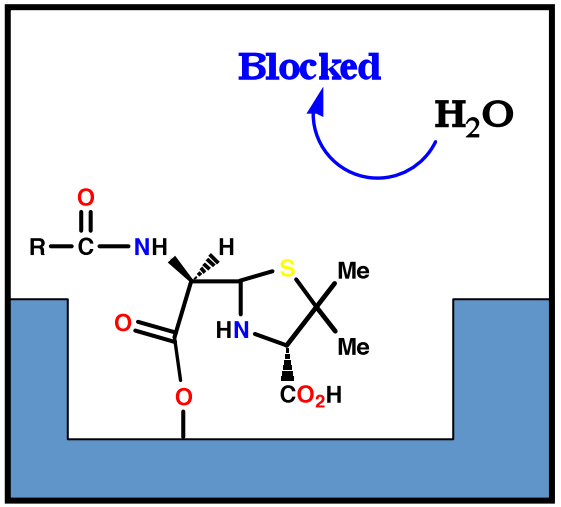


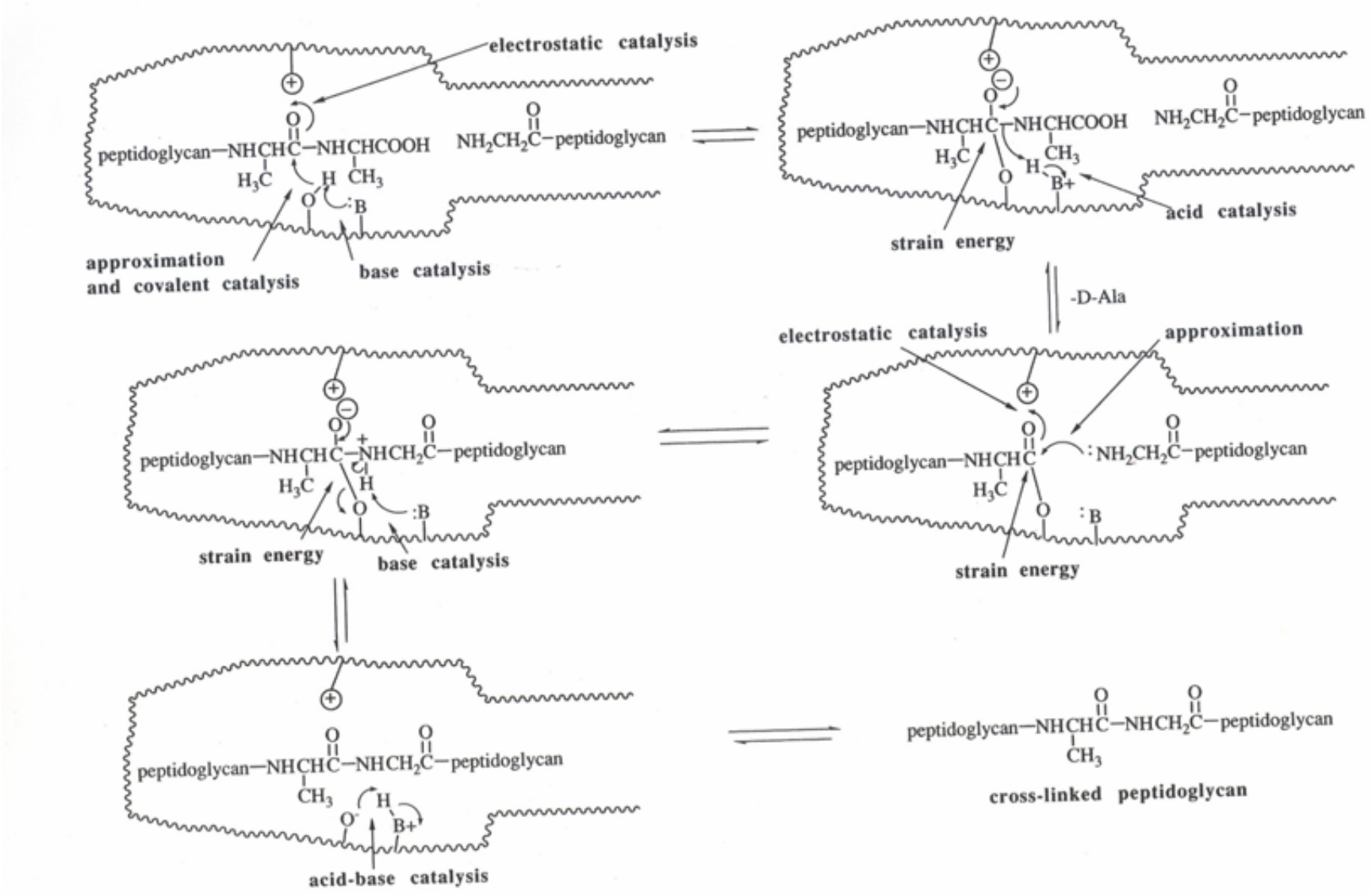


Mechanism inhibited by penicillin:



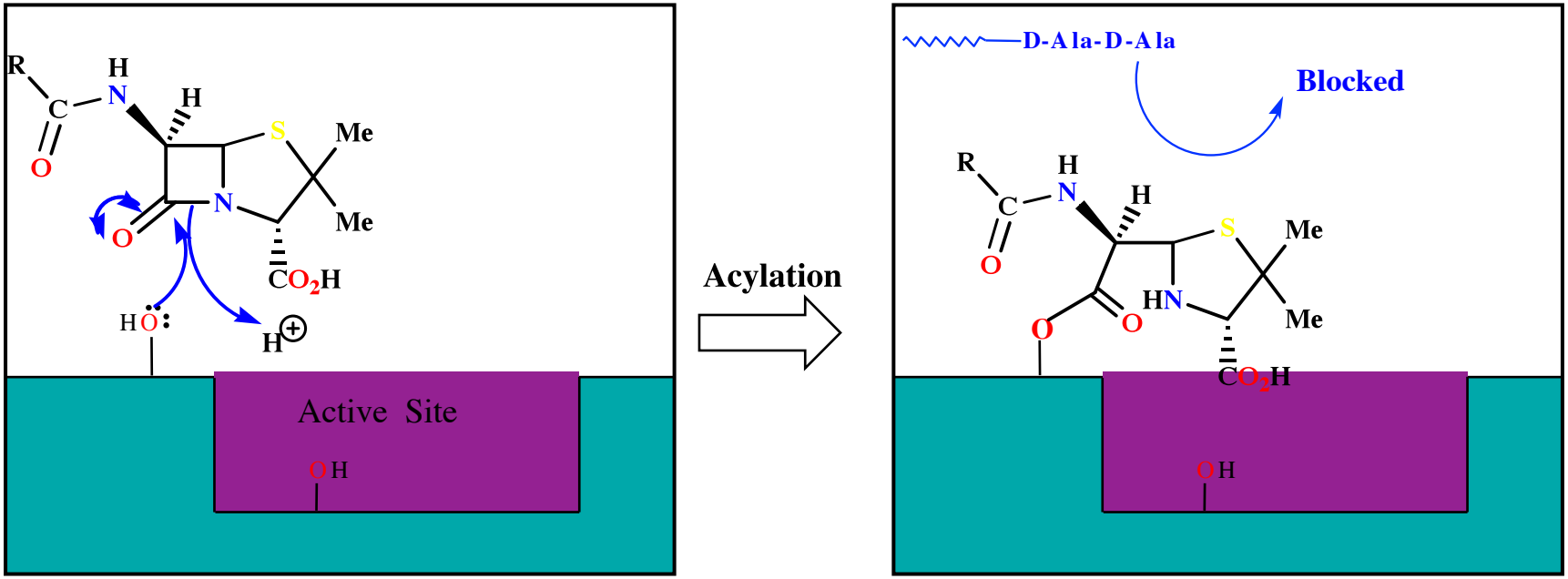






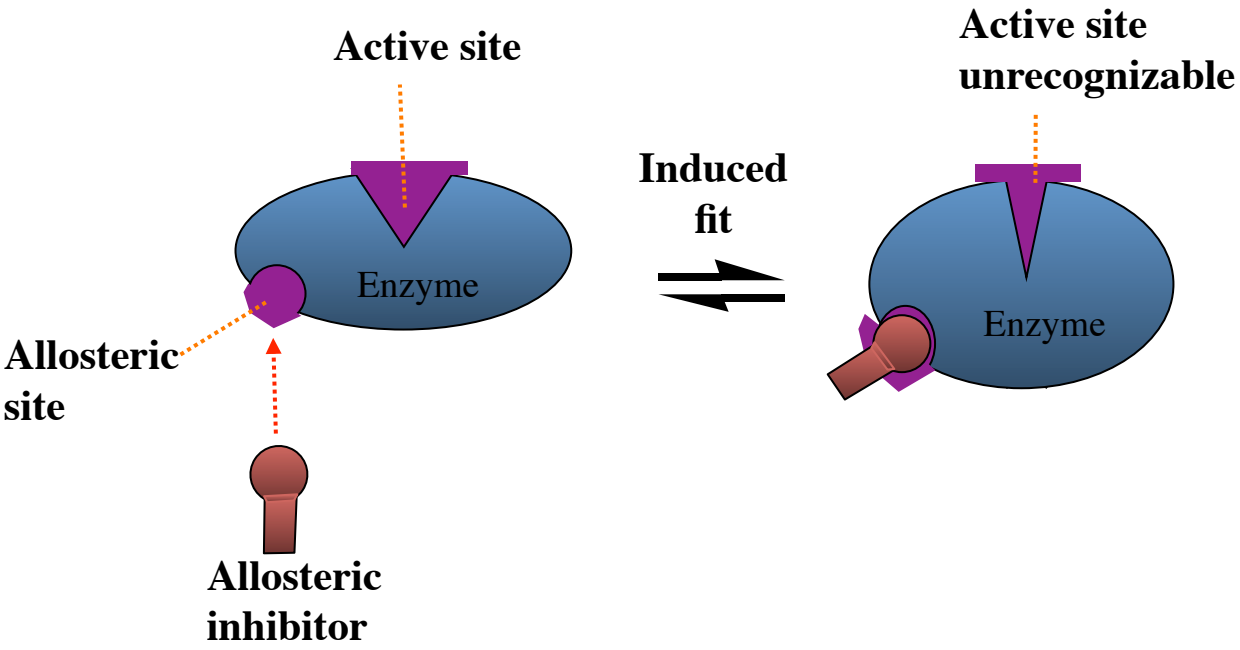
From *The Organic Chemistry of Drug Design and Drug Action* by Richard B. Silverman, Academic Press, 1992.

Alternative “umbrella” mechanism

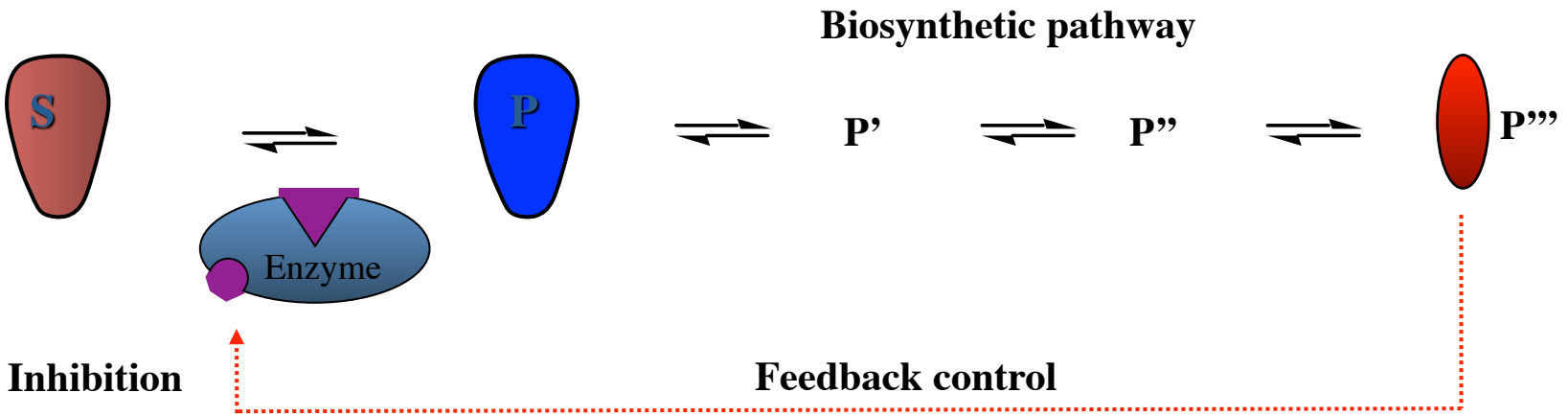


Allosteric inhibition (reversible)

- Inhibitor binds reversibly to allosteric site
- Induced fit alters the tertiary structure of the enzyme
- Active site is distorted and is not recognized by the substrate
- Increasing substrate concentration does not reverse inhibition
- Inhibitor is not similar in structure to the substrate



Allosteric inhibition (reversible)



- Enzymes with allosteric sites often at start of biosynthetic pathways
- Enzyme is controlled by the final product of the pathway
- Final product binds to the allosteric site and switches off enzyme
- Inhibitor may have a similar structure to the final product

Examples

Drugs acting through alteration of enzyme reactions

Substrate	Enzyme	Products	Inhibitor	Uses
Acetylcholine	Acetylcholine esterase	Choline; acetate	Neostigmine	Myasthenia gravis and to reverse neuromuscular block
Arachidonate	Cyclooxygenase	Prostanoids	Aspirin	Heart disease and inflammation
Angiotensin (AT)I	AT converting enzyme	AT II	Captopril	Hypertension, heart failure, post-infarct
Hypoxanthine	Xanthine oxidase	Uric acid	Allopurinol	Gout
HMG-CoA	HMG-CoA reductase	Mevalonic acid	Simvastatin	To lower blood cholesterol
Folate	Dihydrofolate reductase	Tetrahydrofolate	Trimethoprim	With cotrimoxazole as antibacterial
Thymidine	Viral reverse transcriptase		Zidovudine	HIV infection
Deoxyribonucleotides	DNA polymerase	DNA	Cytarabine	Anticancer drug