Topic 3 Proteins as Drug Targets

Enzymes-Chapter 3, 4 Patrick and Part I Corey

Enzymes as Targets

Structure and function of enzymes
- The active site
- Substrate binding
  - Induced fit
  - Bonding forces

Catalysis mechanisms
- Acid/base catalysis
- Nucleophilic residues

Overall process of enzyme catalysis

Competitive (reversible) inhibitors

Non competitive (irreversible) inhibitors

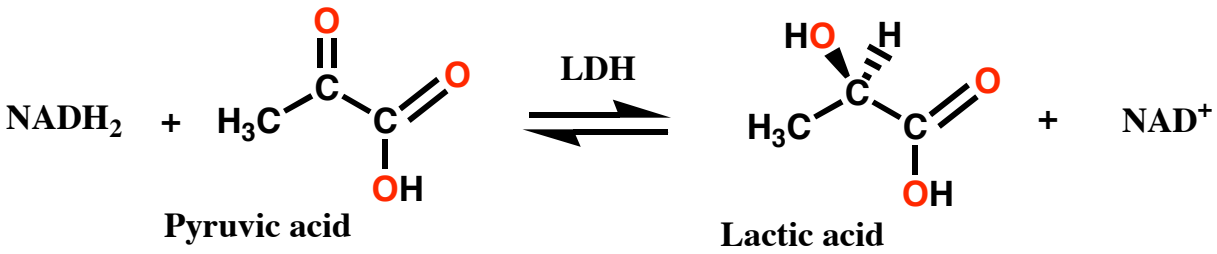
Non competitive (reversible) allosteric inhibitors

Example: NSAIDS for inflammation

Structure and function of enzymes

- Globular proteins acting as the body's catalysts
- Speed up time for reaction to reach equilibrium
- Lower the activation energy of a reaction

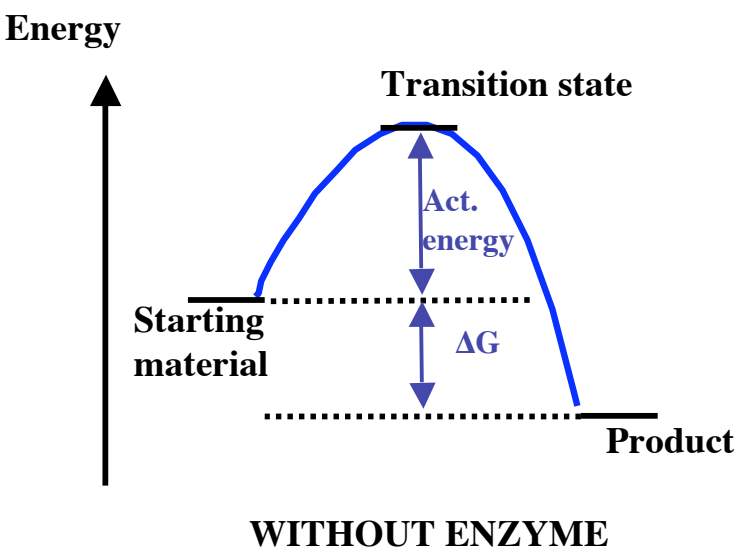
Example:



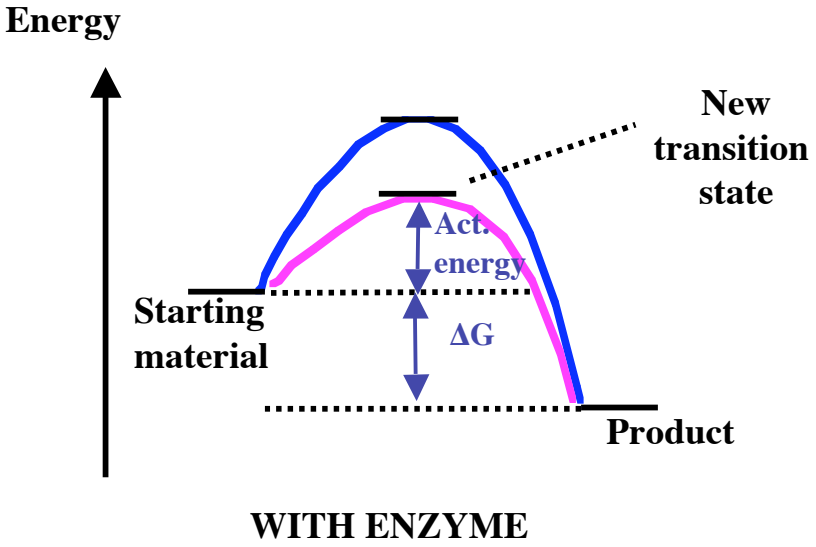
LDH = Lactate dehydrogenase (enzyme)
$NADH_2$ = Nicotinamide adenosine dinucleotide (reducing agent & cofactor)
Pyruvic acid = Substrate

1. Structure and function of enzymes

Lowering the activation energy of reaction



WITHOUT ENZYME



WITH ENZYME

- **Enzymes lower the activation energy of a reaction but ΔG remains the same**

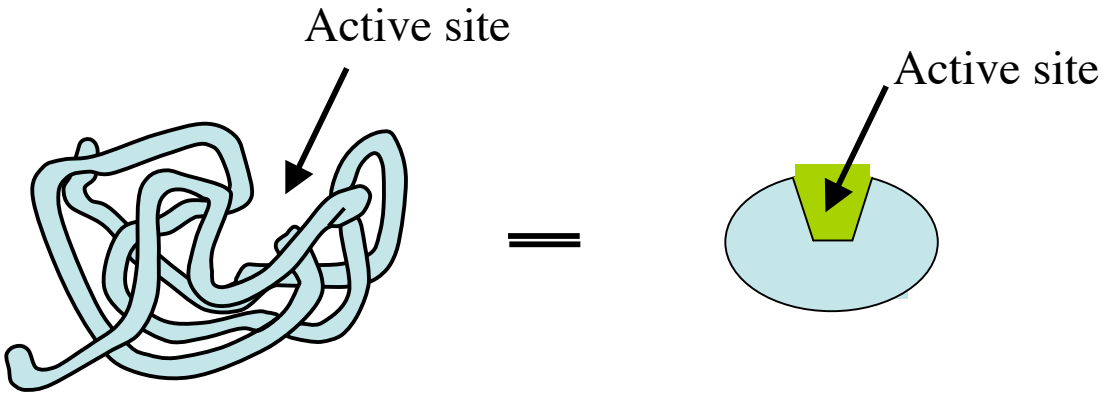
1Structure and function of enzymes

Methods of enzyme catalysis

- **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 (transition state)**
- **Provide acid / base catalysis**
- **Provide nucleophiles**

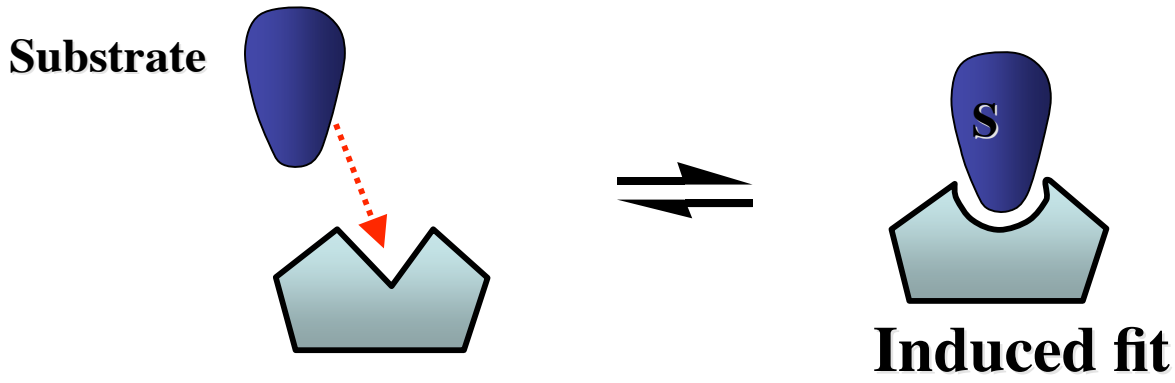
The active site

- **Hydrophobic hollow or cleft on the enzyme surface**
- **Accepts reactants (substrates and cofactors)**
- **Contains amino acids which**
  - bind reactants (substrates and cofactors)
  - catalyse the reaction



Substrate binding

Induced fit



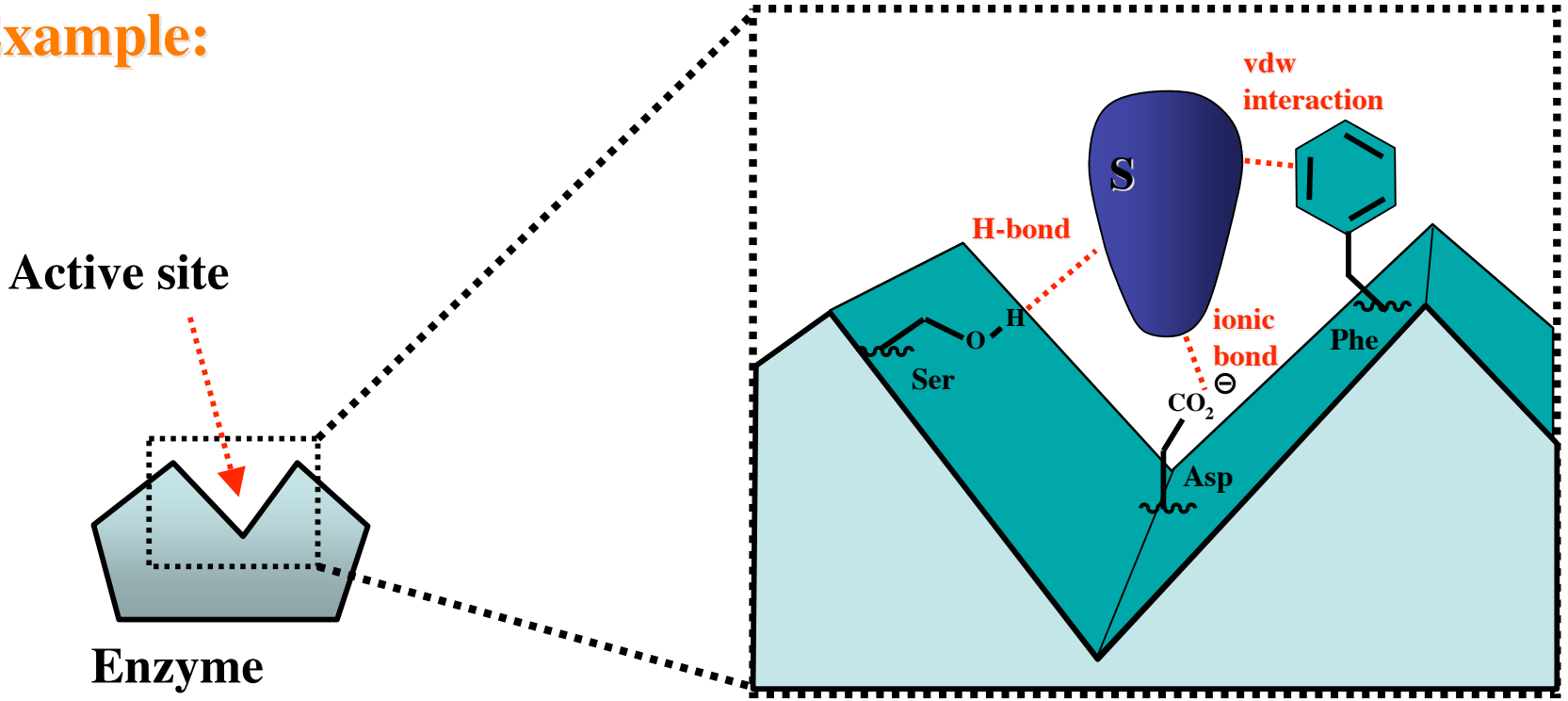
- Active site is nearly the correct shape for the substrate
- Binding alters the shape of the enzyme (induced fit)
- Binding will strain bonds in the substrate
- Binding involves intermolecular bonds between functional groups in the substrate and functional groups in the active site

Substrate binding

Bonding forces

- Ionic
- H-bonding
- van der Waals

Example:

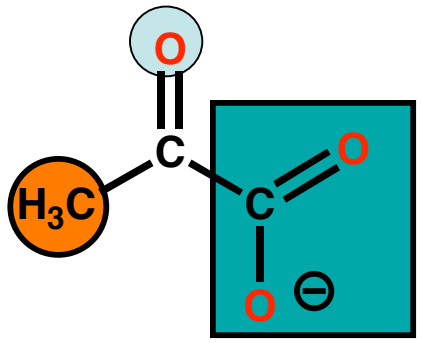


Substrate binding

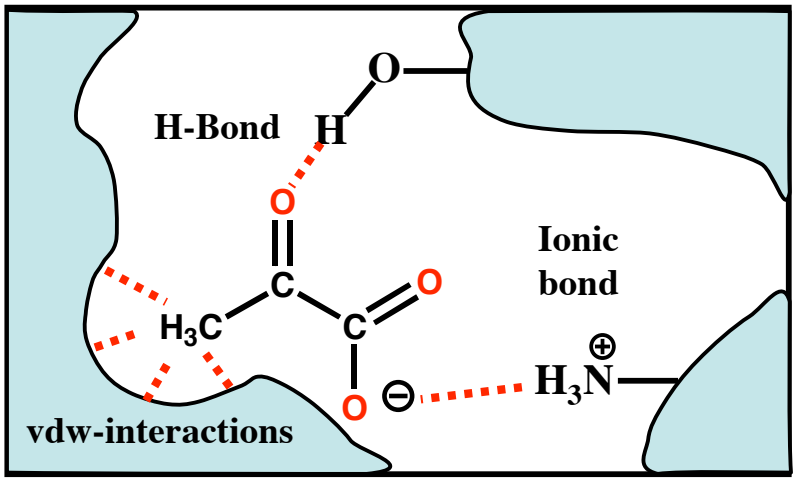
Bonding forces

- Ionic
- H-bonding
- van der Waals

Example: Binding of pyruvic acid in LDH



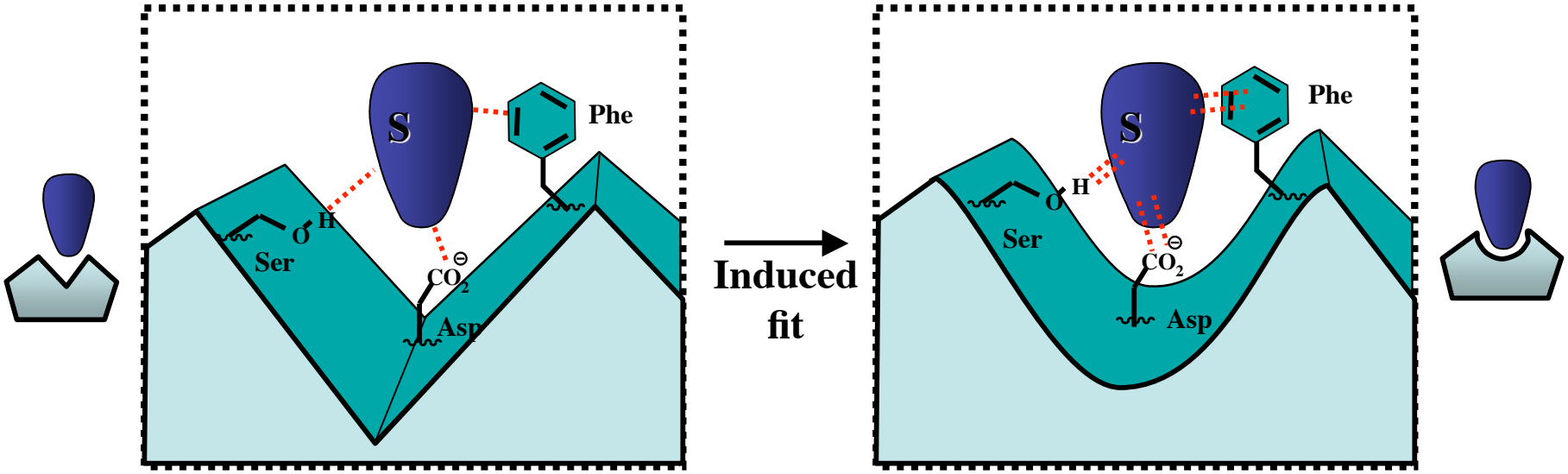
Possible interactions



Substrate binding

Bonding forces

- **Induced fit - Active site alters shape to maximise intermolecular bonding**

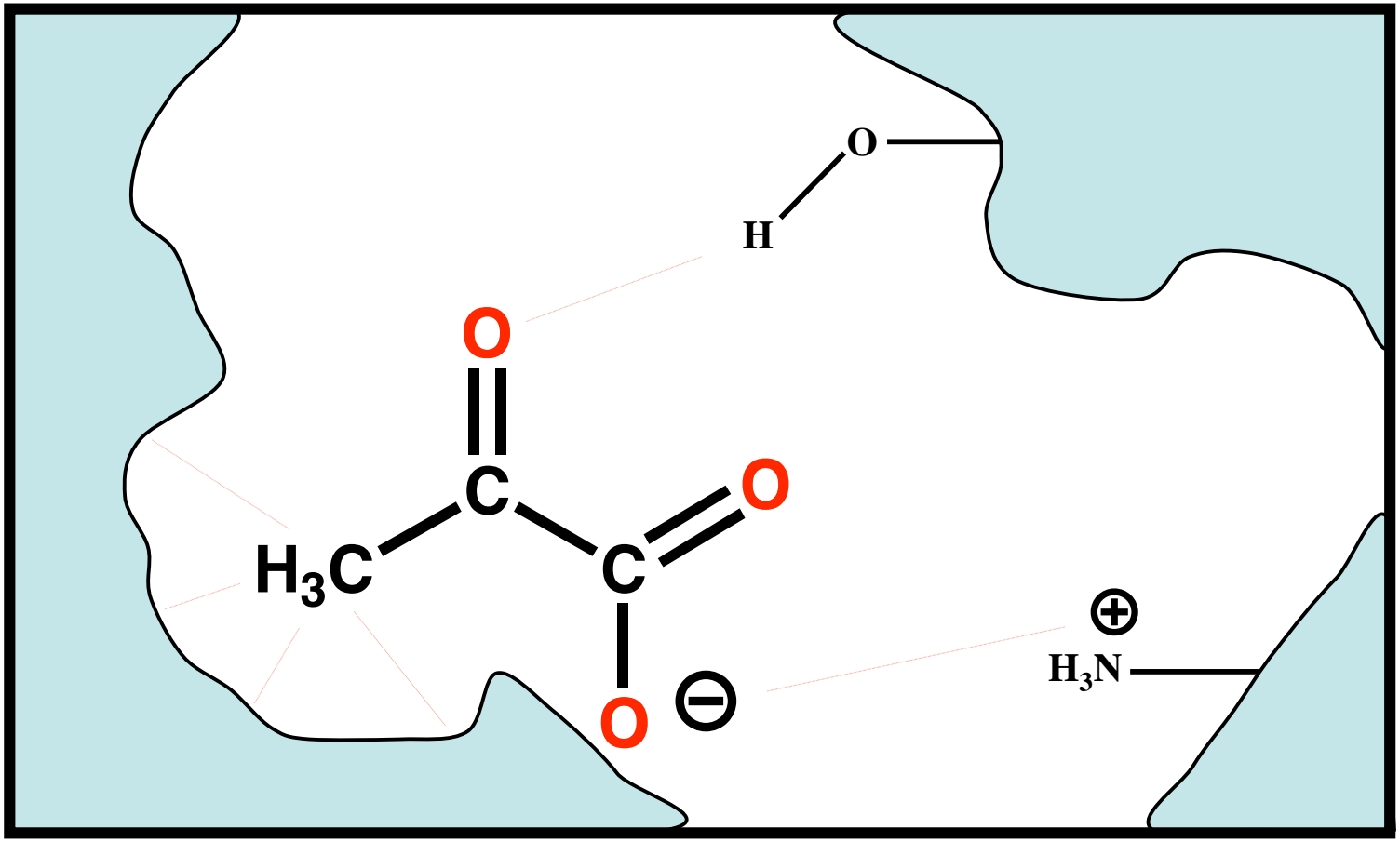


Intermolecular bonds not optimum length for maximum bonding

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

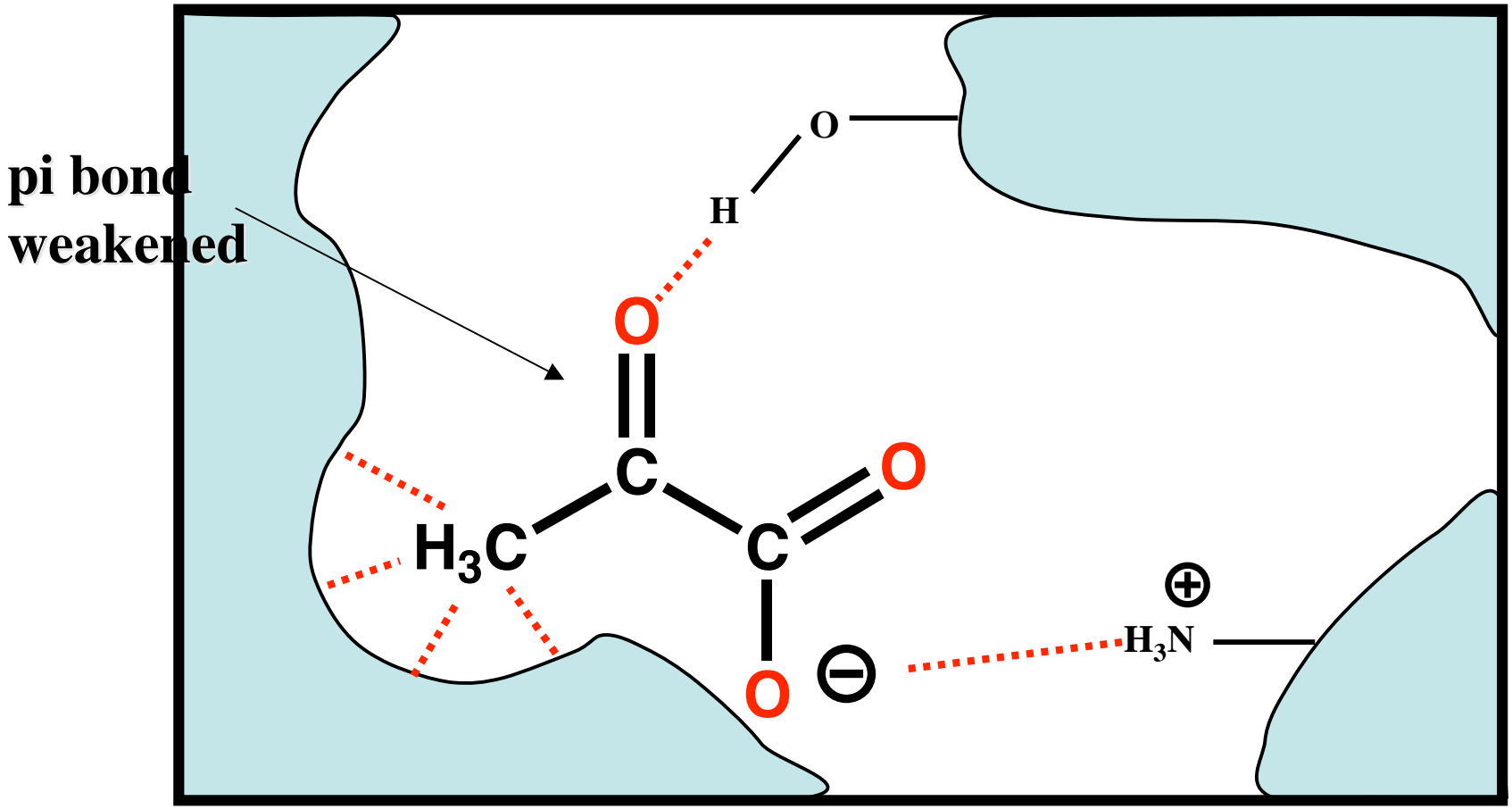
Substrate binding

Example: **Binding of pyruvic acid in LDH**



Substrate binding

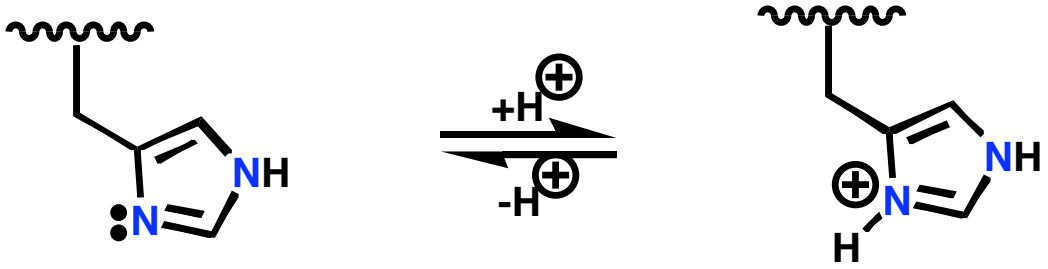
Example: **Binding of pyruvic acid in LDH**



Catalysis mechanisms

4.1 Acid/base catalysis

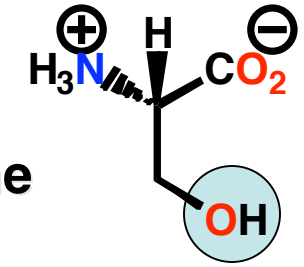
- **Histidine**



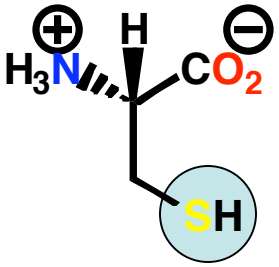
Non-ionised
Acts as a basic catalyst
(proton 'sink')

Ionised
Acts as an acid catalyst
(proton source)

4.2 Nucleophilic residues



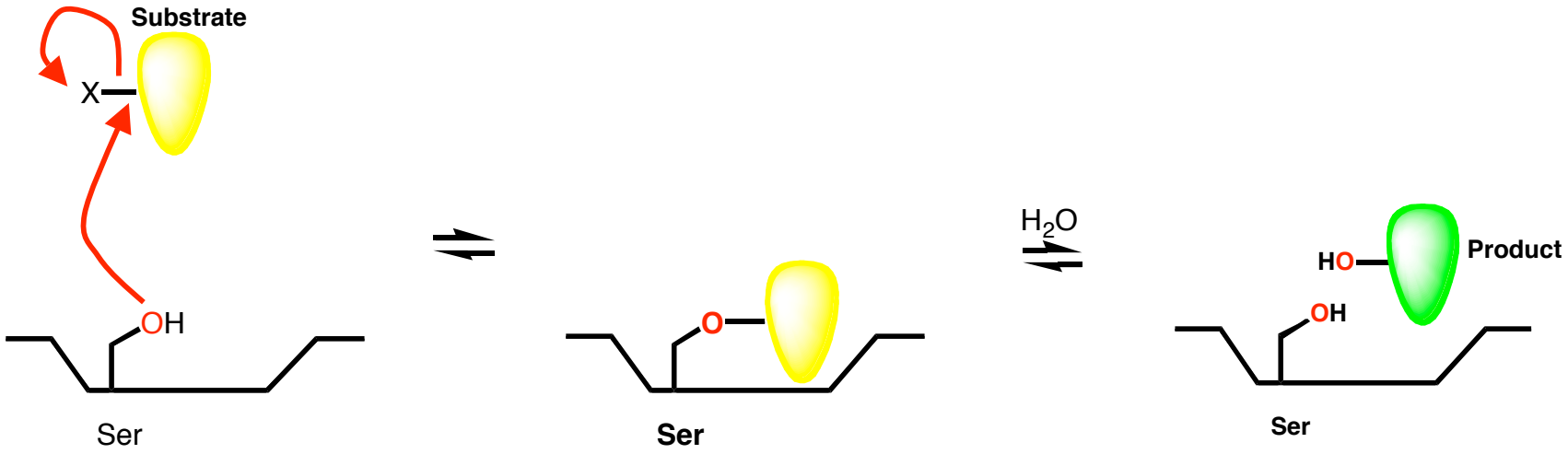
L-Serine



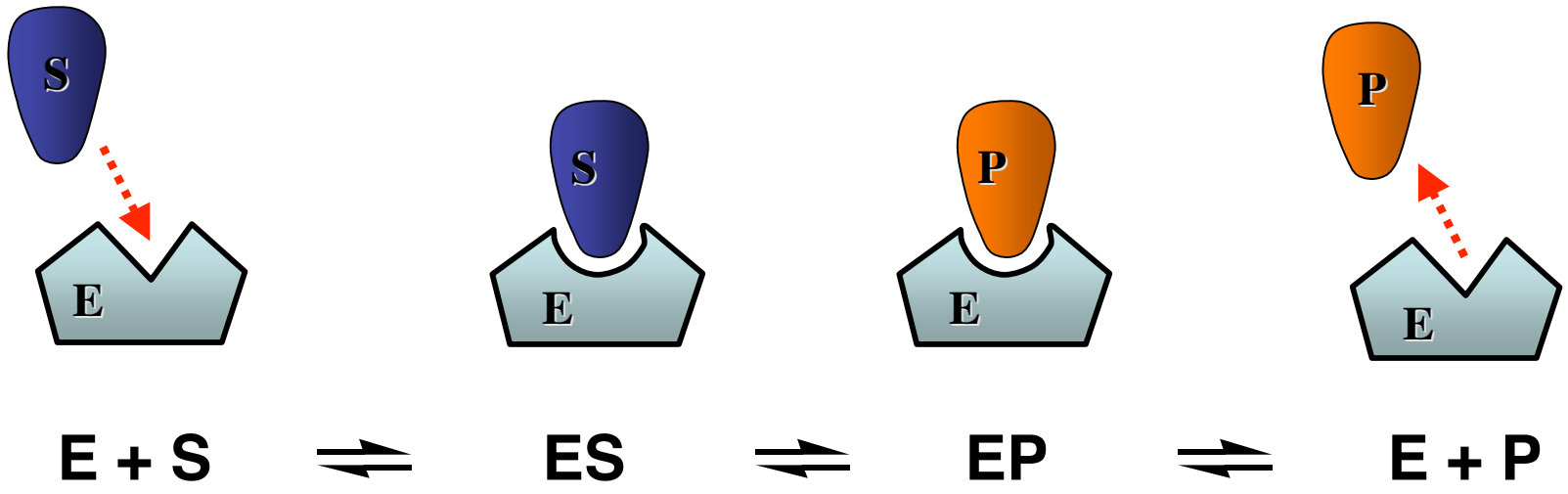
L-Cysteine

Catalysis mechanisms

Serine acting as a nucleophile

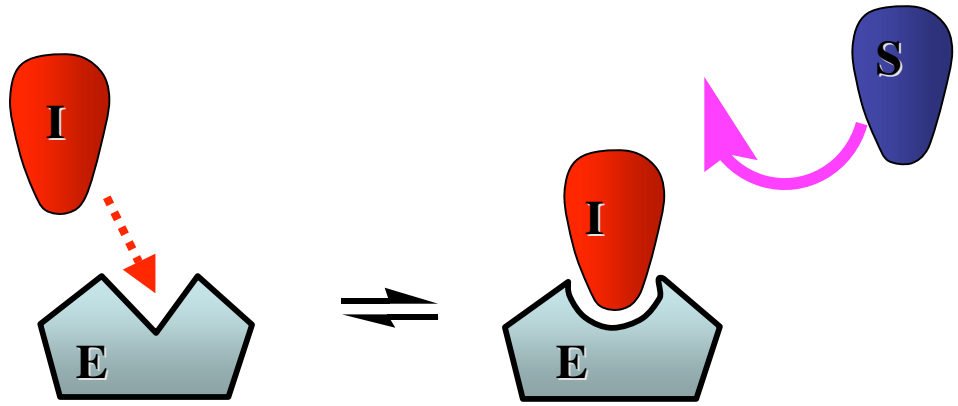


Overall process of enzyme catalysis



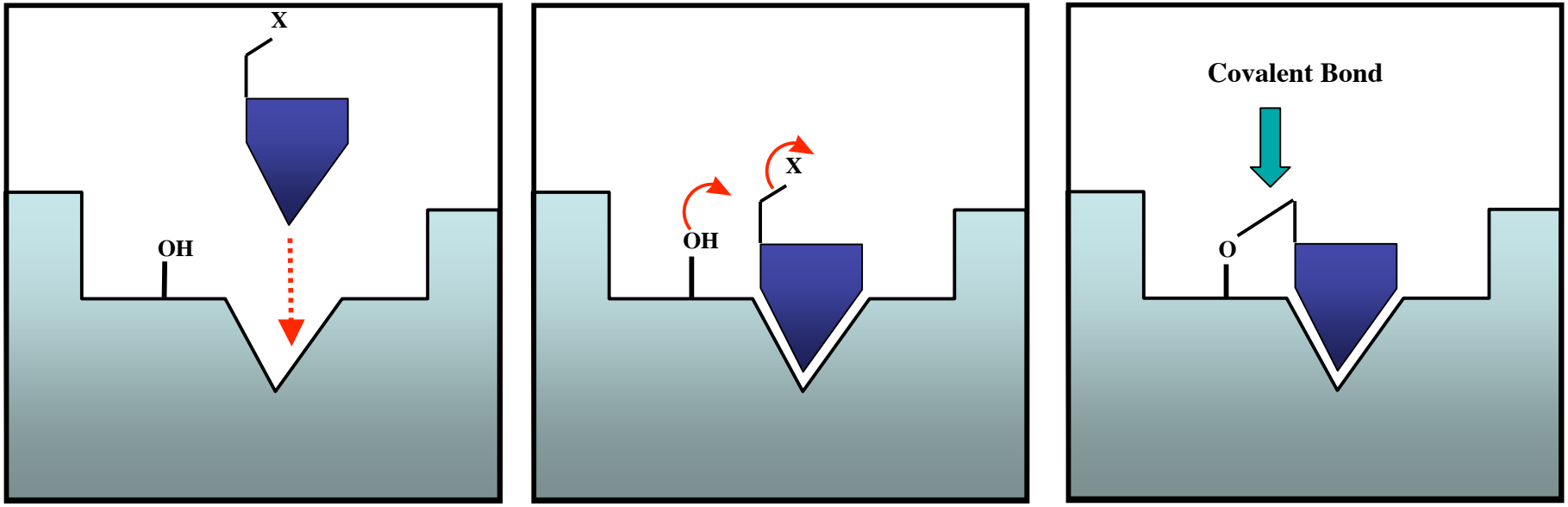
- **Binding interactions must be;**
  - strong enough to hold the substrate sufficiently long for the reaction to occur
  - weak enough to allow the product to depart
- **Implies a fine balance**
- **Drug design - designing molecules with stronger binding interactions results in enzyme inhibitors which block the active site**

Competitive (reversible) inhibitors



- Inhibitor binds reversibly to the active site
- Intermolecular bonds are involved in binding
- 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

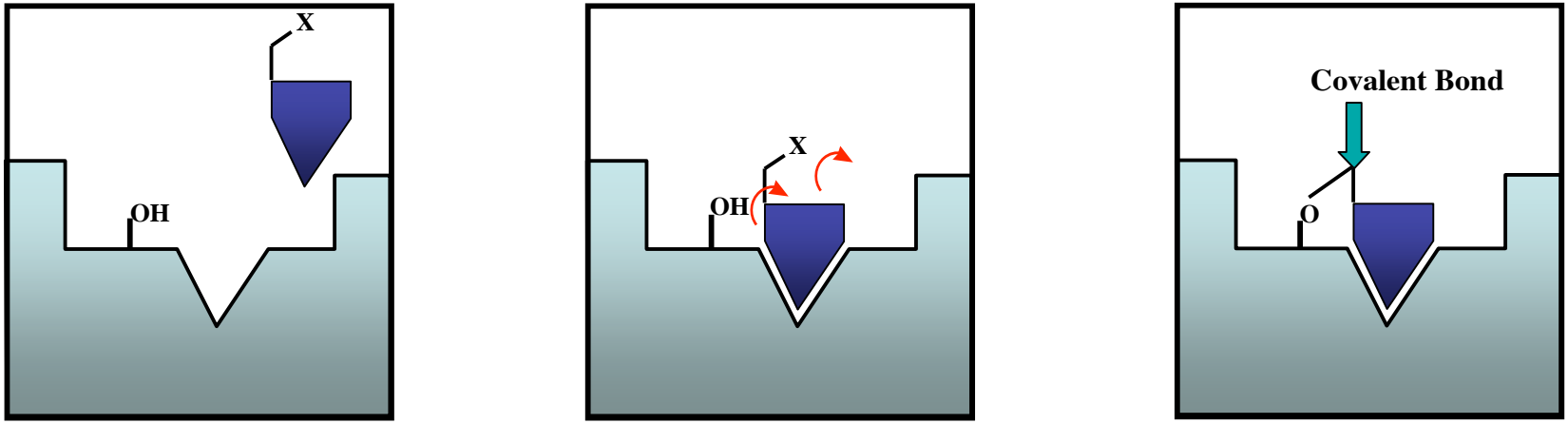
Non competitive (irreversible) inhibitors



Irreversible inhibition

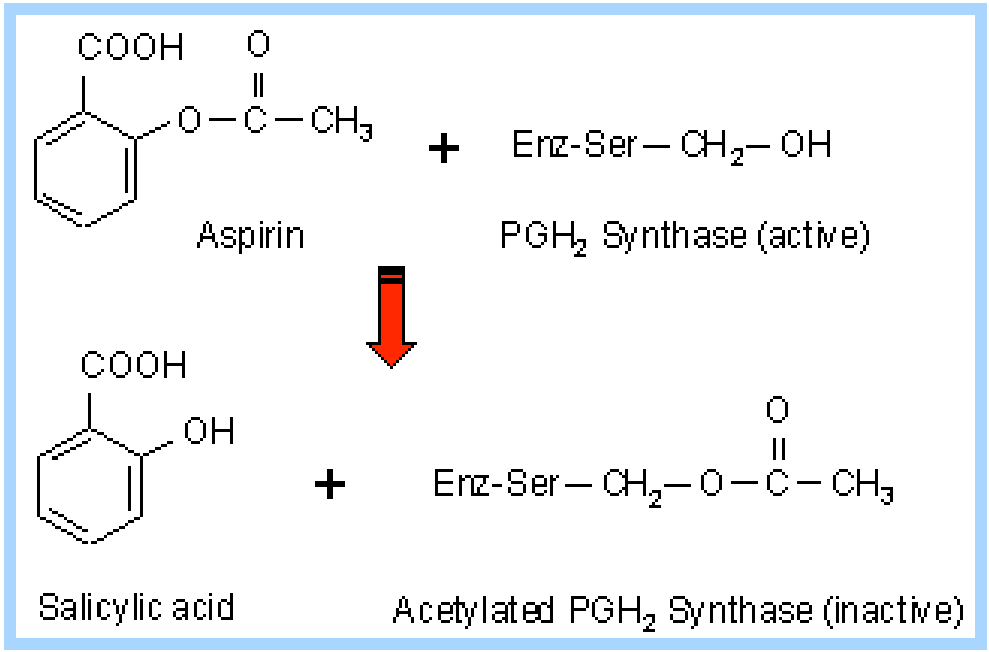
- Inhibitor binds irreversibly to the active site
- Covalent bond formed between the drug and the enzyme
- Substrate is blocked from the active site
- Increasing substrate concentration does not reverse inhibition
- Inhibitor likely to be similar in structure to the substrate

Non competitive (irreversible) inhibitors

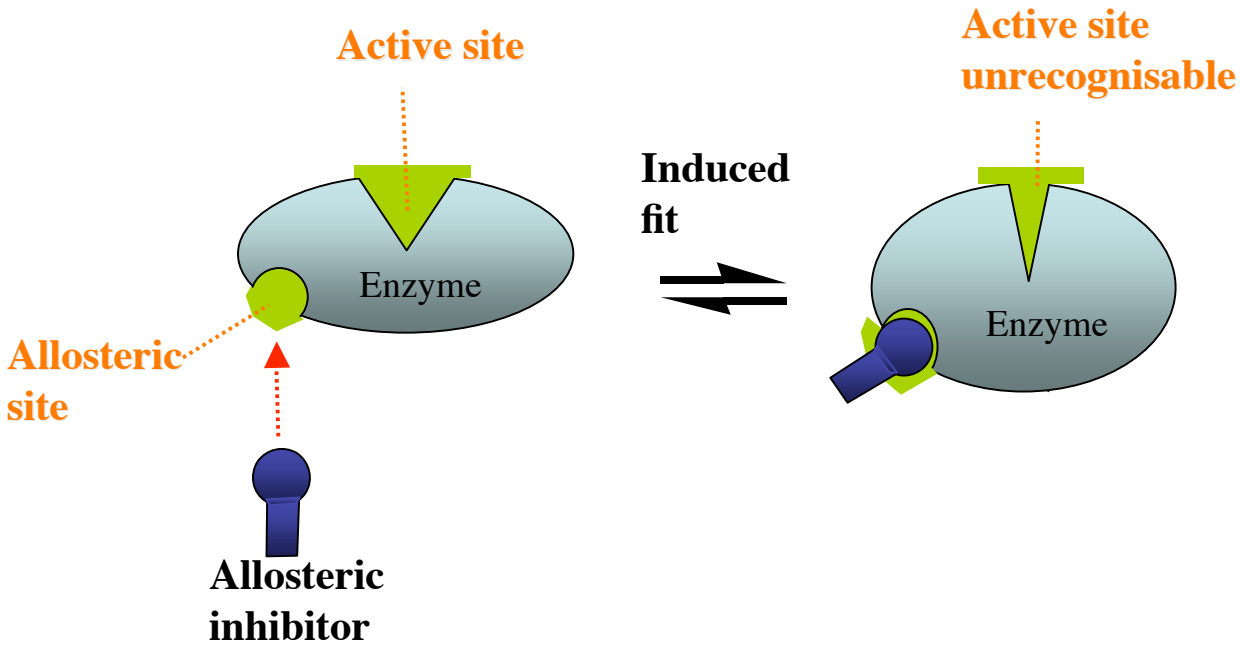


Irreversible inhibition

Example: aspirin for COX (PGH_2 synthase)

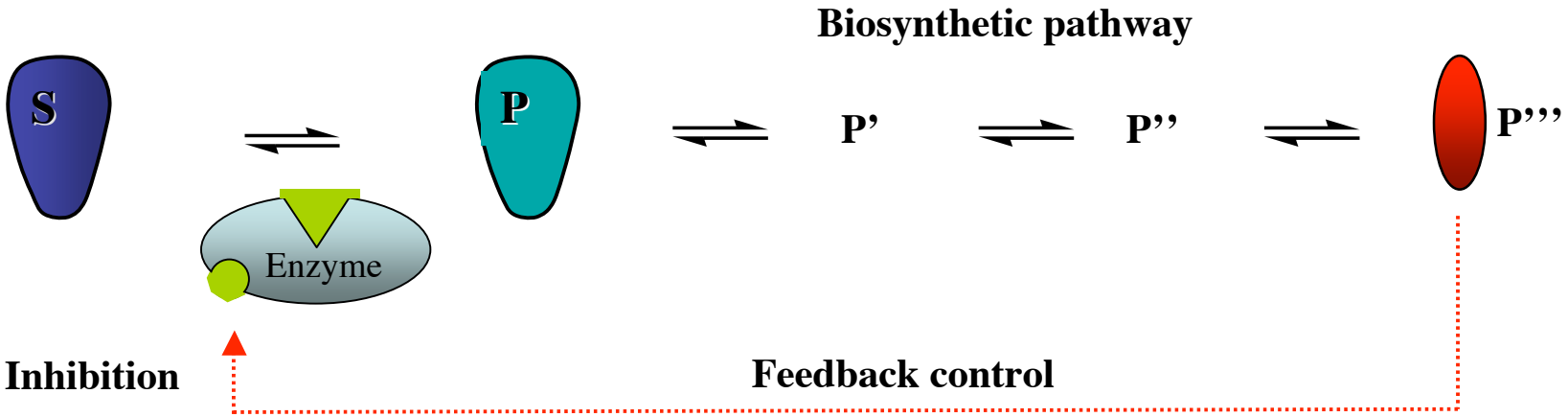


Non competitive (reversible) allosteric inhibitors



- Inhibitor binds reversibly to the allosteric site
- Intermolecular bonds are formed
- Induced fit alters the shape of the enzyme
- Active site is distorted and is not recognised by the substrate
- Increasing substrate concentration does not reverse inhibition
- Inhibitor is not similar in structure to the substrate

Non competitive (reversible) allosteric inhibitors

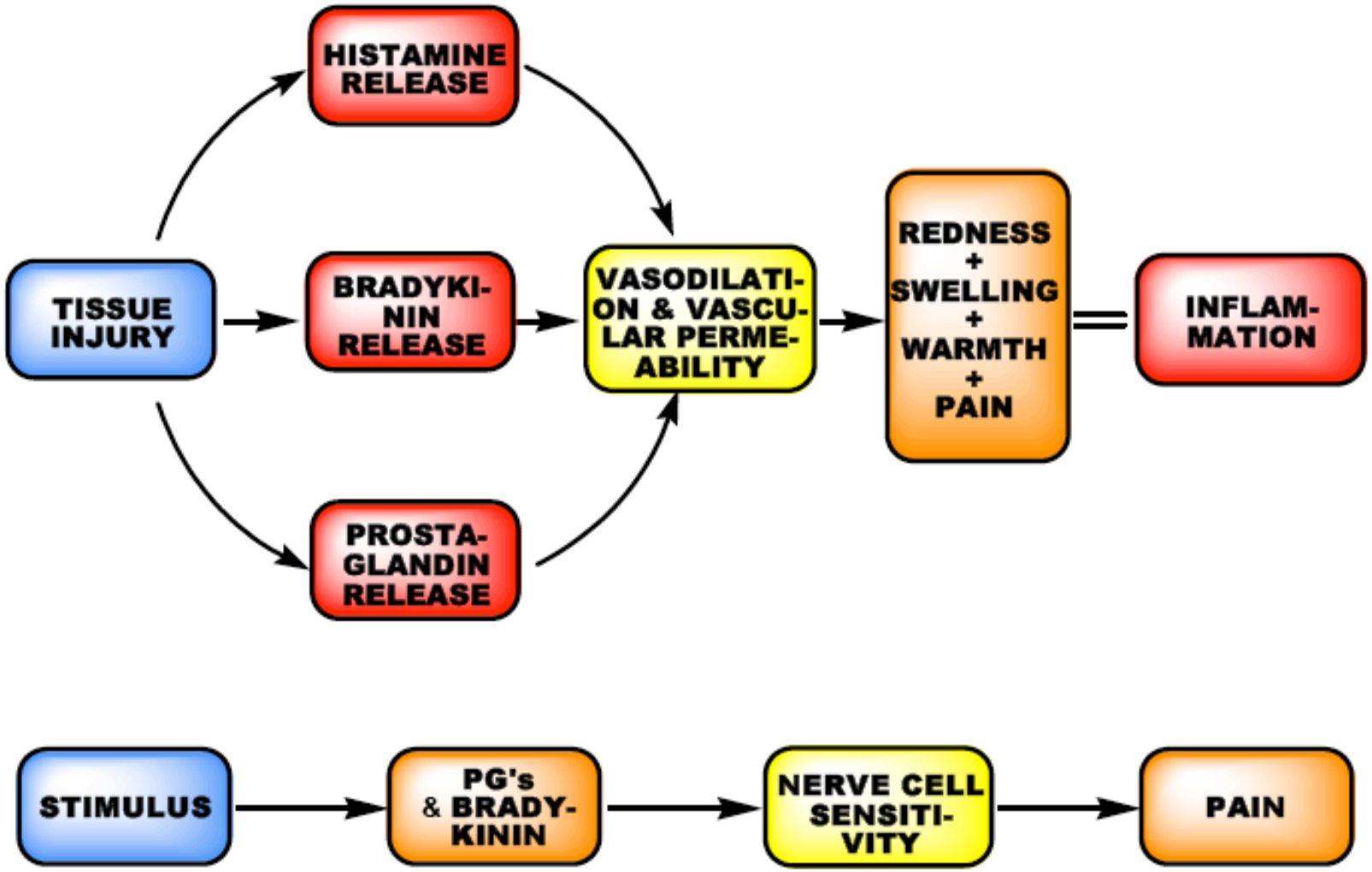


- **Enzymes with allosteric sites often at start of biosynthetic pathways**
- **Enzyme is controlled by the final product of the pathway**
- **Final product binds to the allosteric site and switches off enzyme**
- **Inhibitor may have a similar structure to the final product**

EXAMPLE: NSAIDS for inflammation

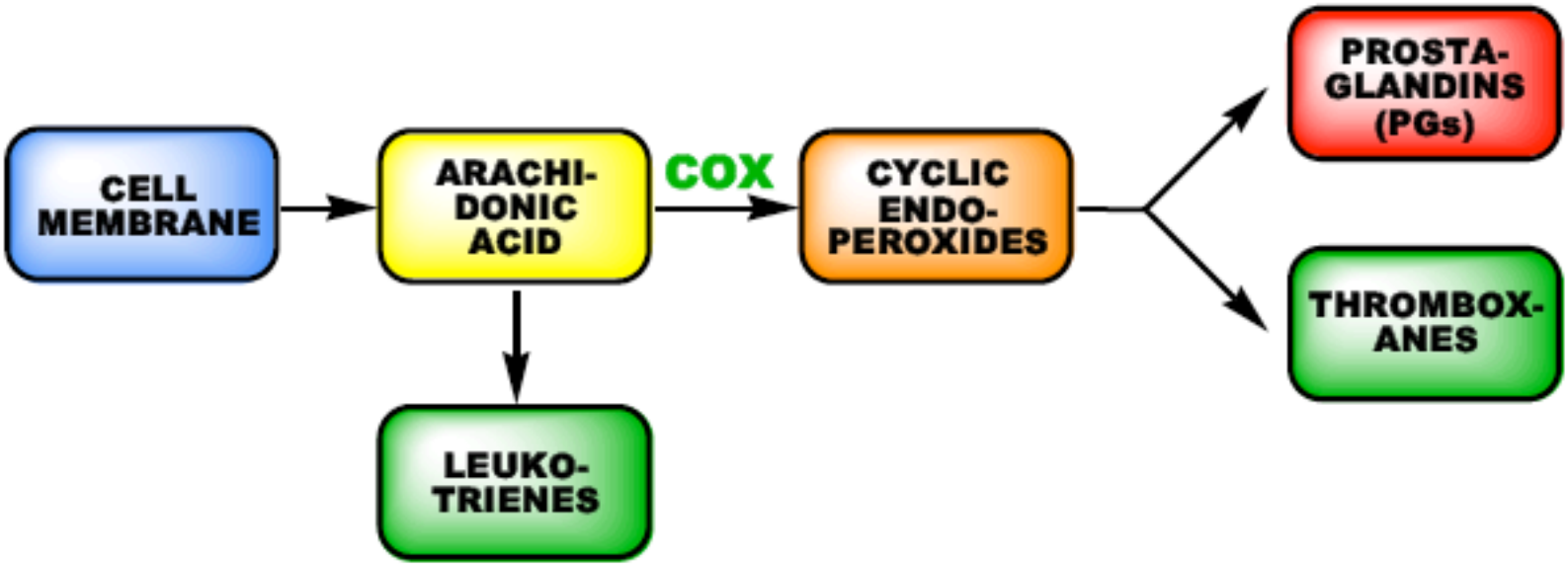
Inflammatory, Cardiovascular and Metabolic Diseases

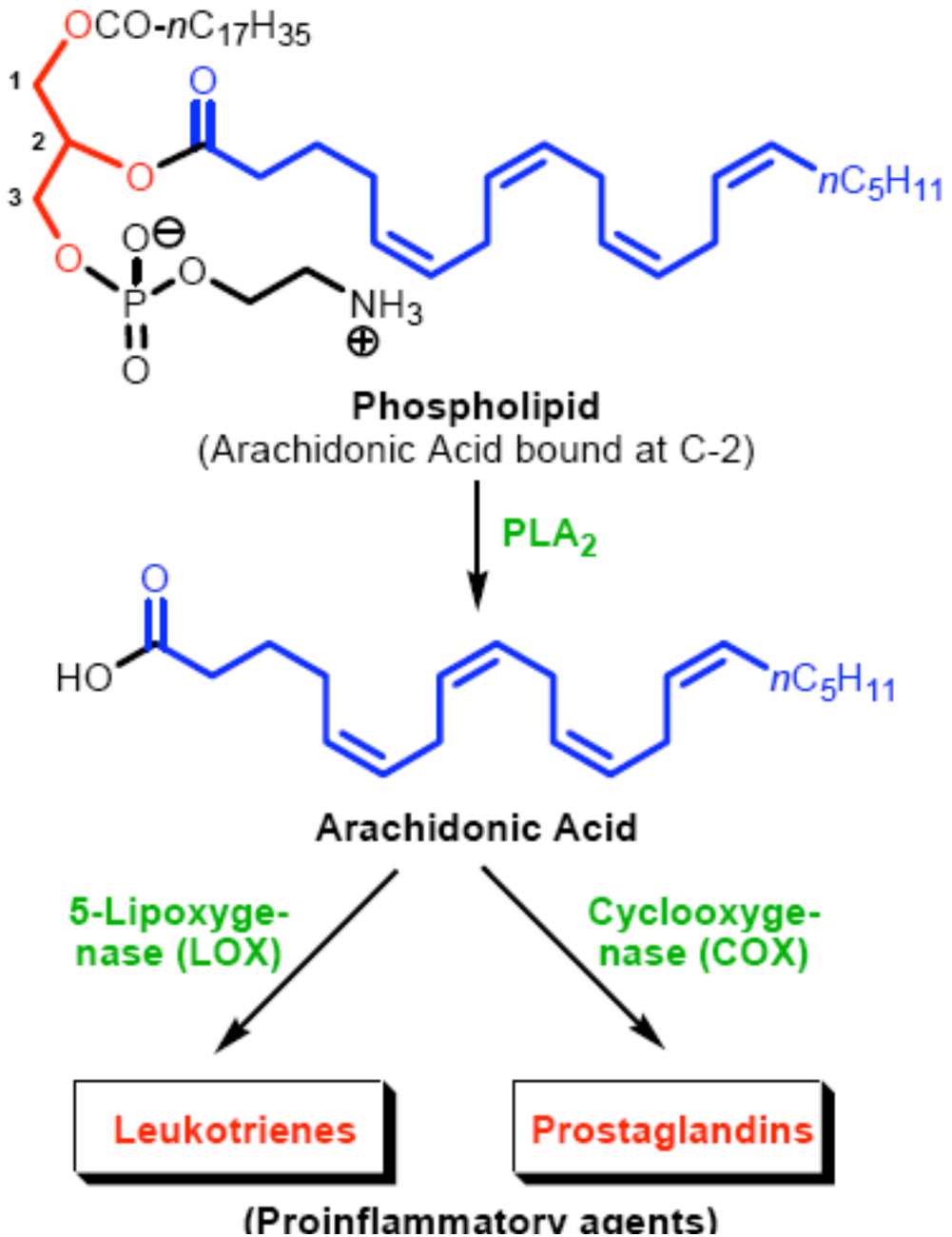
An Overview of Inflammation II.



Inflammatory, Cardiovascular and Metabolic Diseases

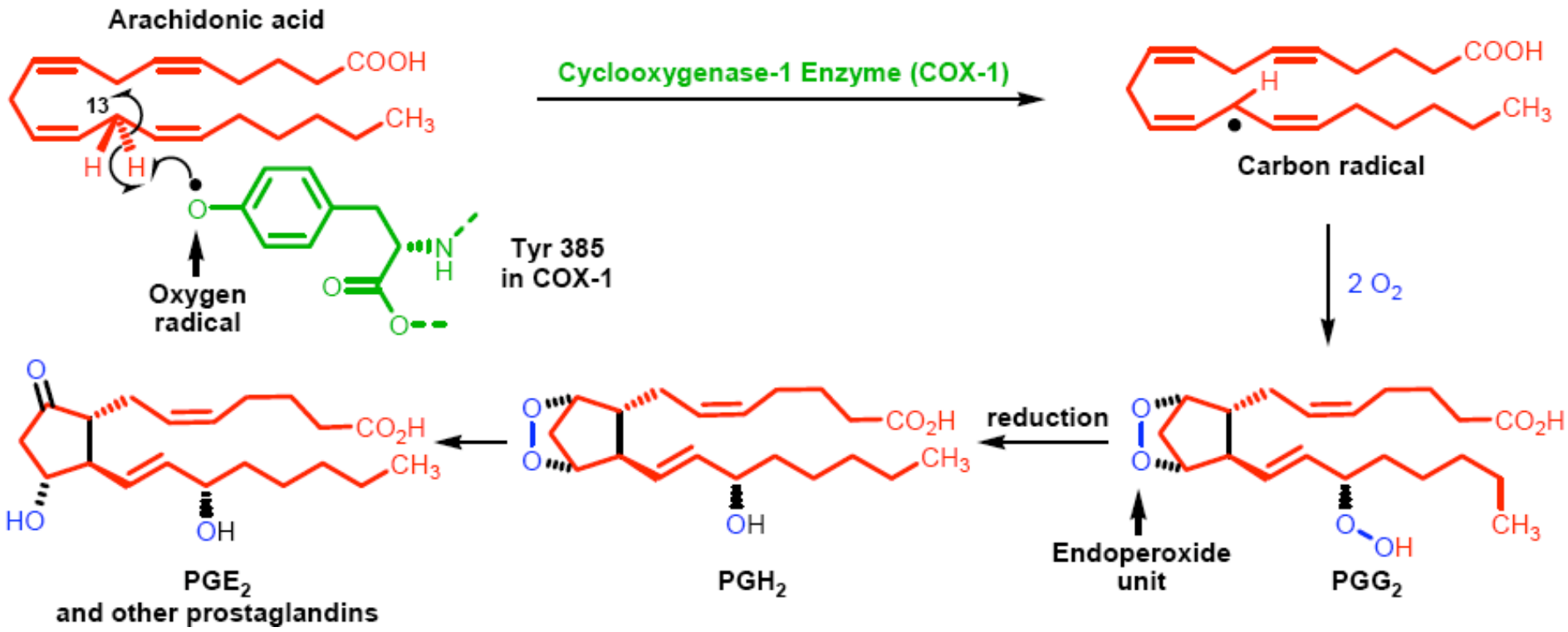
An Overview of Inflammation I.




OCO-nC17H35
1
2
3
O
O
O
O⊖
P
O
O
NH3
⊕
nC5H11
Phospholipid
(Arachidonic Acid bound at C-2)
PLA2
O
HO
nC5H11
Arachidonic Acid
5-Lipoxyge-
nase (LOX)
Cyclooxyge-
nase (COX)
Leukotrienes
Prostaglandins
(Proinflammatory agents)

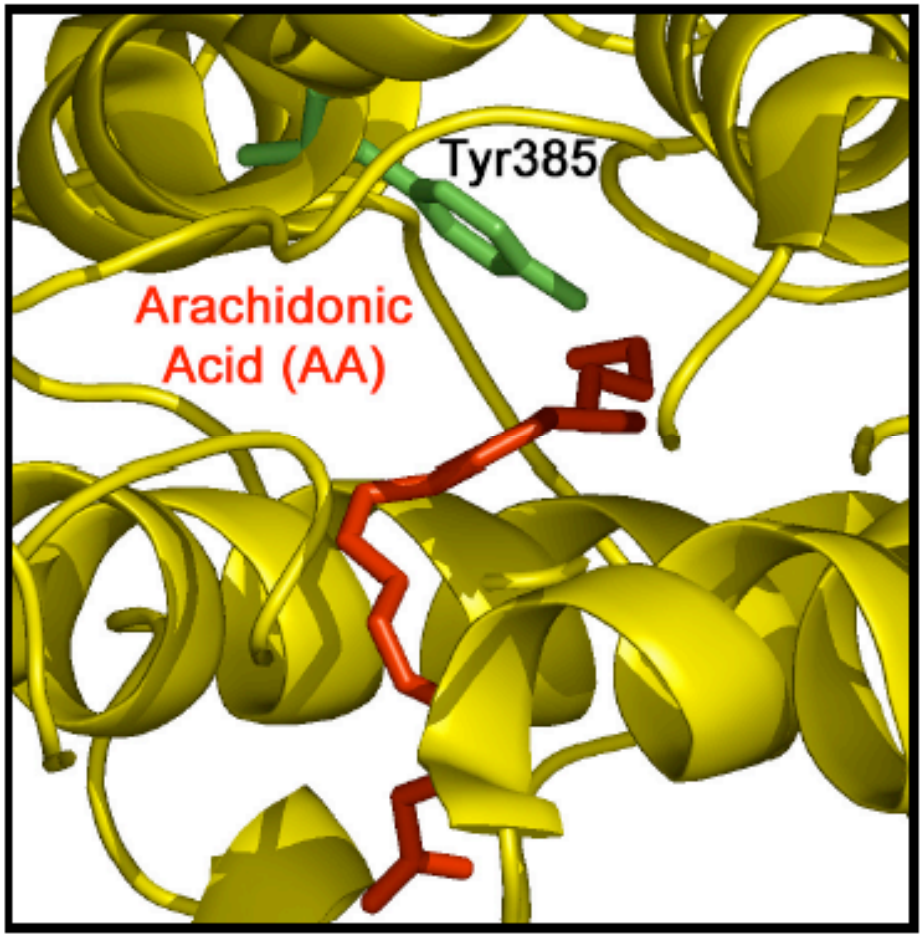
Inflammatory, Cardiovascular and Metabolic Diseases

How Do Anti-Inflammatory Drugs Work? I.

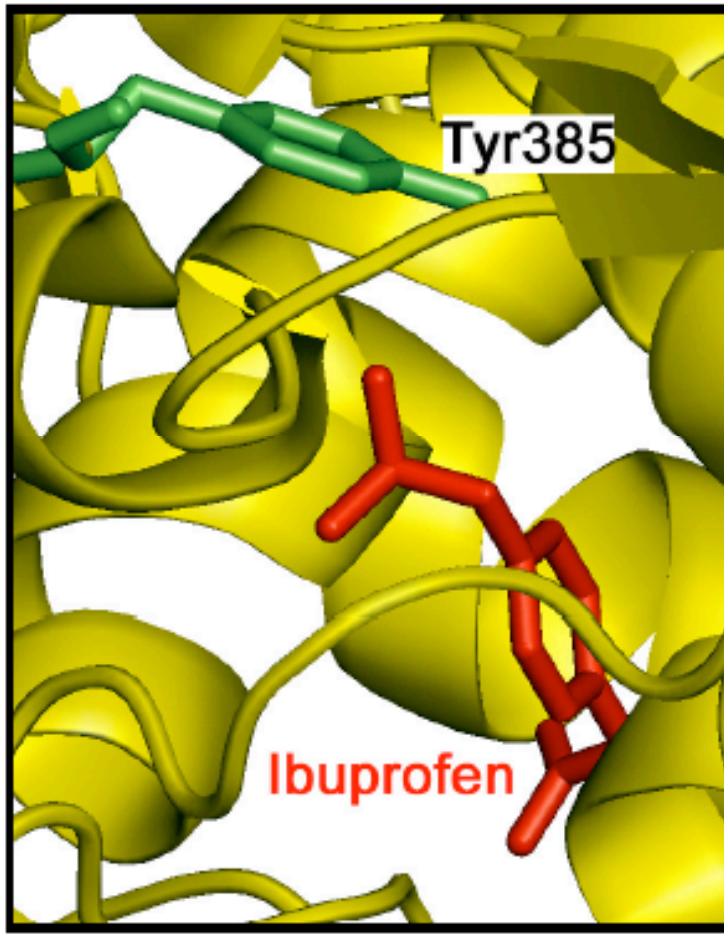


Inflammatory, Cardiovascular and Metabolic Diseases

How Do Anti-Inflammatory Drugs Work? II.



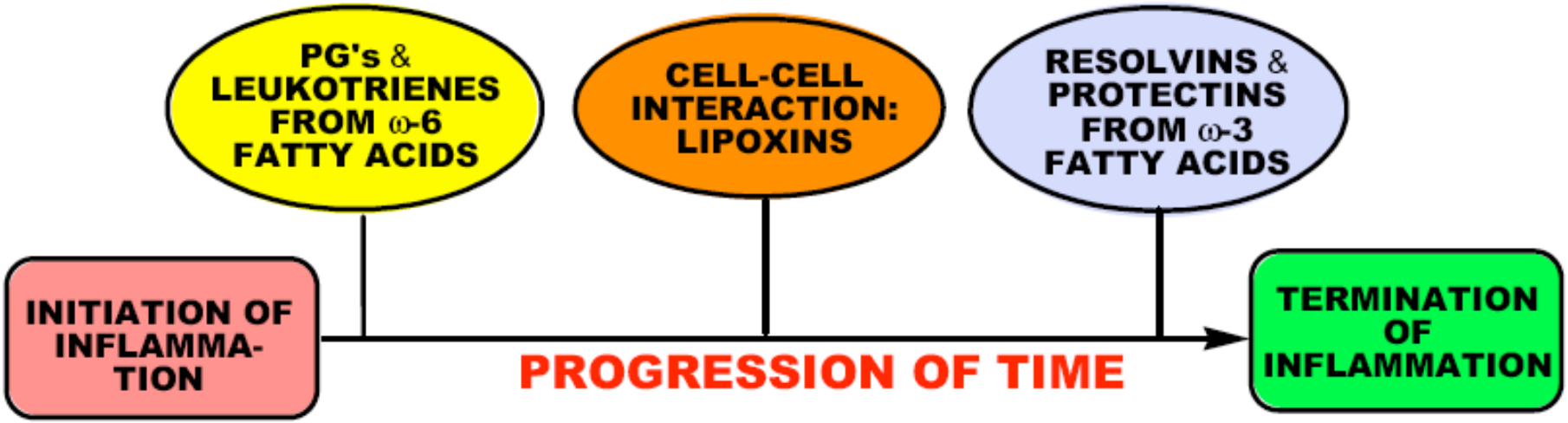
A. Arachidonic Acid in the Active



B. Ibuprofen in the Active

Inflammatory, Cardiovascular and Metabolic Diseases

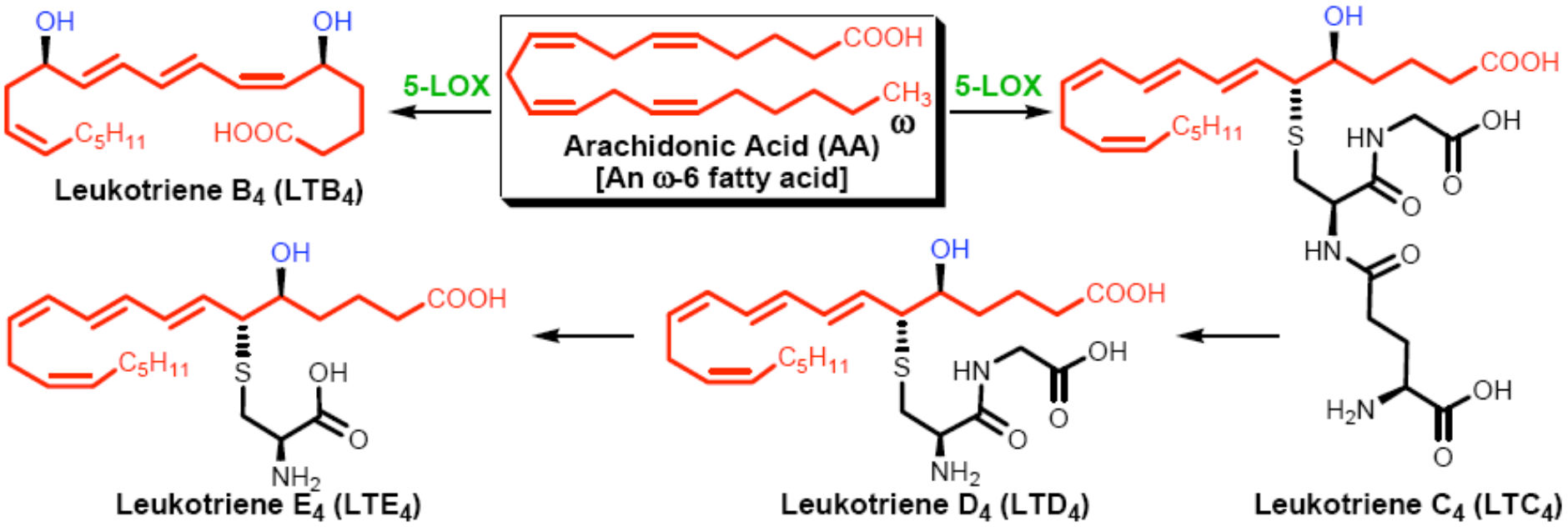
Other Eicosanoids in Inflammation I.



Mediators of Inflammation

Inflammatory, Cardiovascular and Metabolic Diseases

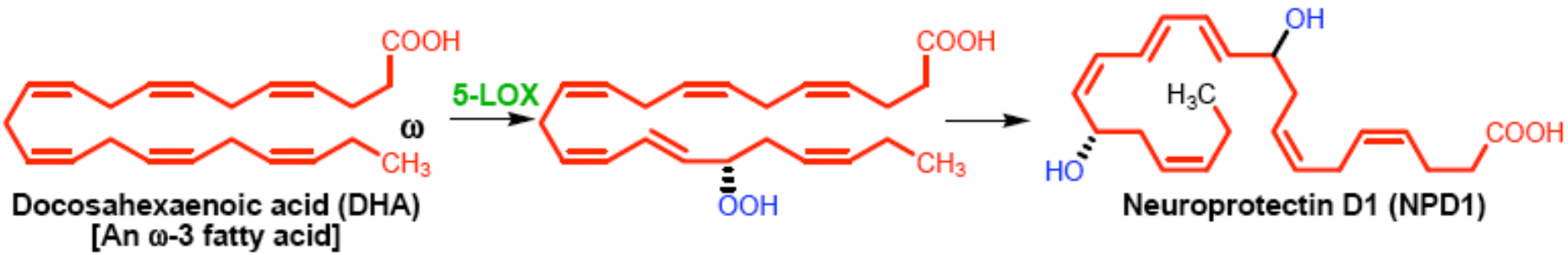
Other Eicosanoids in Inflammation II.



Formation of Various Leukotrienes from Arachidonic Acid *via* 5-Lipoxygenase

Inflammatory, Cardiovascular and Metabolic Diseases

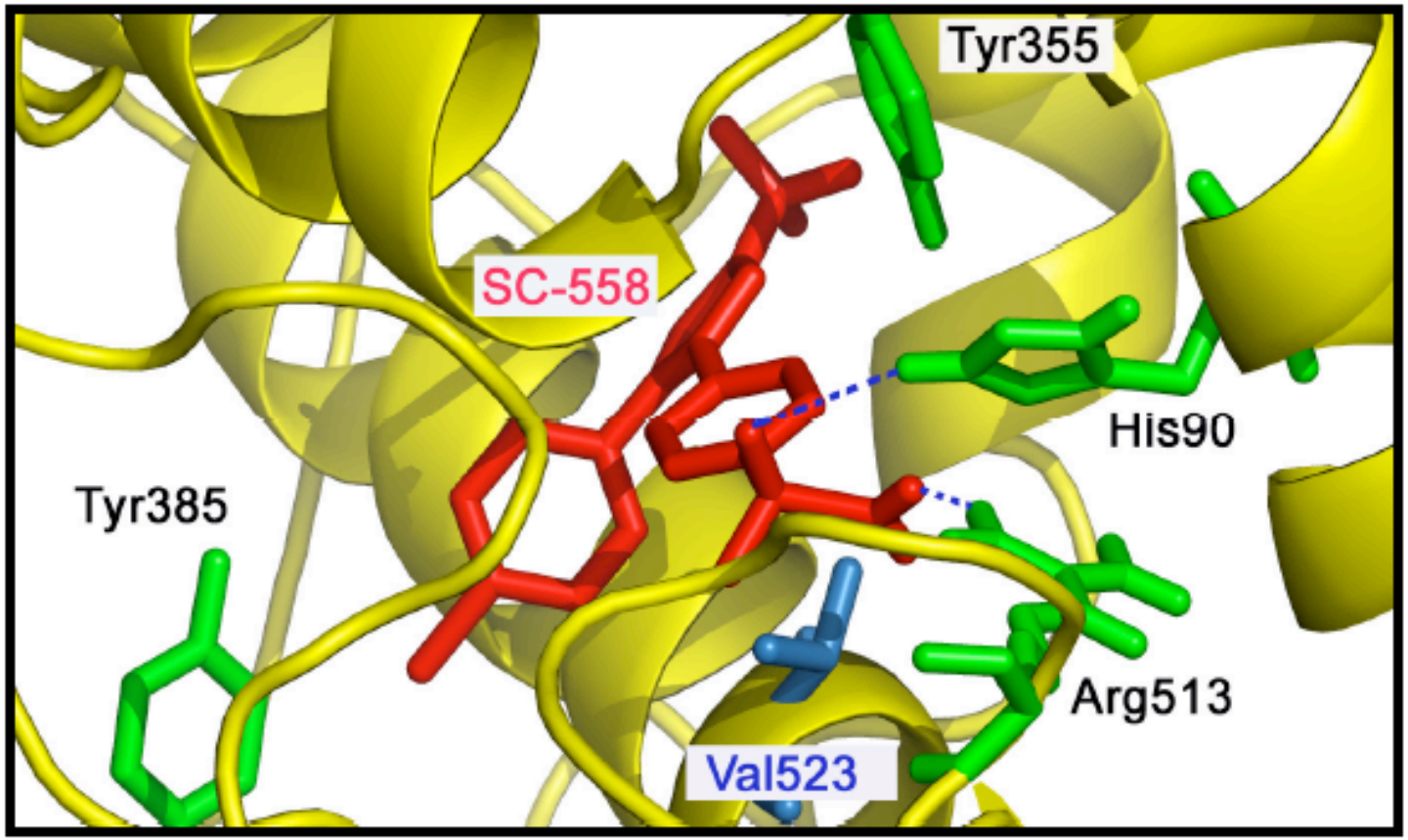
Other Eicosanoids in Inflammation III.



Formation of Neuroprotectin D1 from Docosahexaenoic Acid *via* 5-Lipoxygenase

Inflammatory, Cardiovascular and Metabolic Diseases

Anti-Inflammatory Agents – Celecoxib (Celebrex™) III.



The picture above shows a close structural relative of celecoxib, SC-558, bound in the active site of COX-2. The selectivity results because the phenylsulfonyl group binds in a pocket (formed from His90, Arg513 and Val523) that is not available in COX-1 since it is occupied by a bulky isoleucine side chain rather than the smaller isopropyl group of valine (Val523). The carboxyl group of rofecoxib interacts not with Arg513 but with a different residue, Arg120.