

## Enzyme Substrate Interactions

### Substrate Binding and Enzyme Action

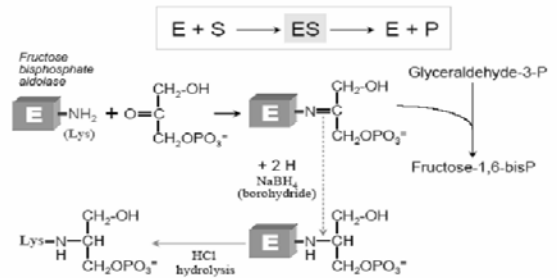
- The first step in an enzyme catalyzed reaction is the formation of the enzyme-substrate complex. This is represented by the equation:



- The region of the enzyme where the substrate binds is called the **active site**. This consists of a substrate binding site and the catalytic site.
- The active site is usually a cleft or pocket created by the unique tertiary structure of the enzyme protein
- Enzyme specificity is due to specificity of substrate binding driven by substrate and enzyme 3D structure
- The ES complex is stabilized in the transition state by non-covalent interactions between substrate and the amino acid in the active site.

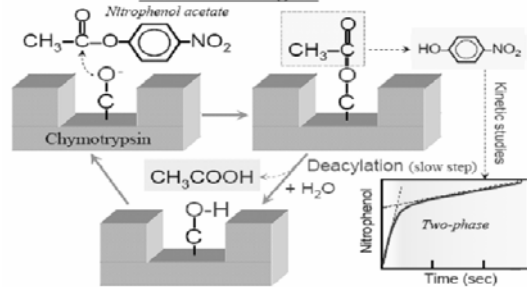
## Identification of Enzyme Catalytic Site

### Trapping the enzyme-substrate complex



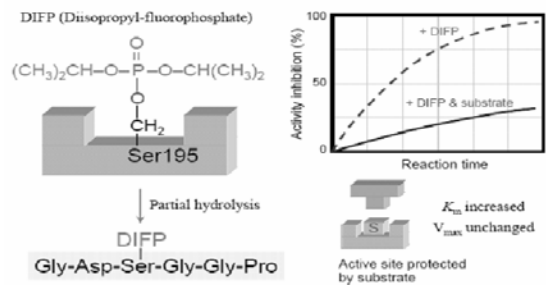
## Identification of Enzyme Catalytic Site

### The use of substrate analogues



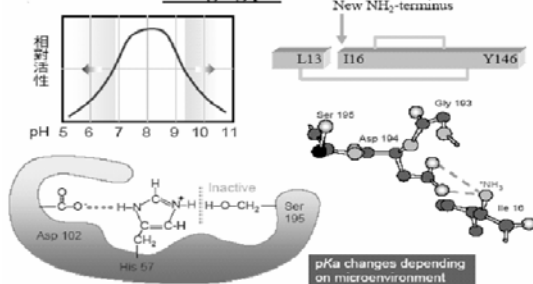
## Identification of Enzyme Catalytic Site

### Chemical modification protected by substrate



## Identification of Enzyme Catalytic Site

### The effect of changing pH



## Enzyme Substrate Interactions

### Factors Affecting Reaction Rates

The study of enzyme reaction rates is called enzyme kinetics. Enzyme kinetics are affected by:

- Temp and pH:**
  - Each enzymatic reaction has an optimum pH and optimum temperature. Extreme temp or pH disrupts enzyme structure and therefore reaction rate
- Enzyme concentration:**
  - At saturating substrate concentration, the initial velocity is directly related to the enzyme concentration.  $E + S = ES = E + P$ . Thus, as long as S is not limiting, more E leads to more ES.
- Substrate concentration:**
  - The reaction rate =  $k [P] / [S]$ . The rate can be increased by adding more substrate, or by removing product as it is formed

## Enzyme Action

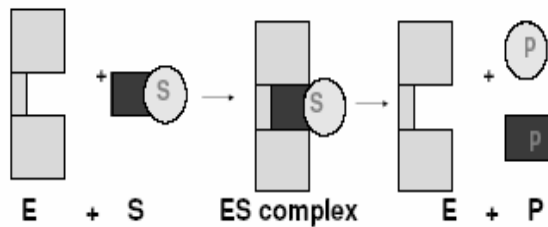
- An enzyme binds a substrate in a region called the active site
- Only certain substrates can fit the active site
- Amino acid R groups in the active site help substrate bind
- Enzyme-substrate complex forms
- Substrate reacts to form product
- Product is released

## Mode of Action

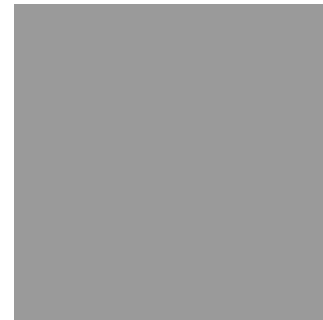
### Models of Enzyme-Substrate Binding

- **Lock and key model.** This means that the enzyme's tertiary structure consists of a unique pocket or site which is tailor-made to fit only its substrate and nothing else, just as a key fits into a lock.
- **Induced-fit model:** This updated model states that enzymes interact with substrates and in the process change their conformation such that the enzyme is snug around the substrate, sort of like a glove around a hand.

## Enzyme Action - Lock & Key Model



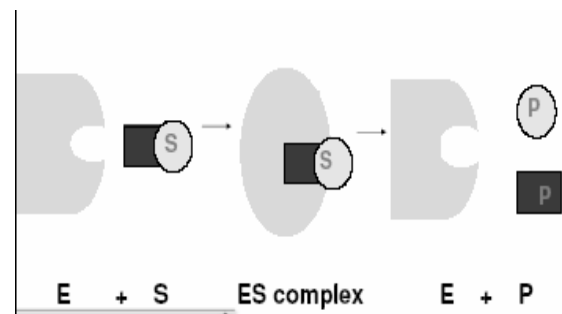
## Enzyme Action - Lock & Key Model



## Enzyme Substrate Interactions

- The favored model of enzyme substrate interaction is known as the induced fit model.
- This model proposes that the initial interaction between enzyme and substrate is relatively weak,
  - These weak interactions rapidly induce conformational changes in the enzyme that strengthen binding and bring catalytic sites close to substrate bonds to be altered.
- After binding takes place, one or more mechanisms of catalysis generates transition - state complexes and reaction products.
- The possible mechanisms of catalysis are four in number

## Enzyme Action - Induced Fit Model



## Enzyme Substrate Interactions

### Catalysis by Bond Strain:

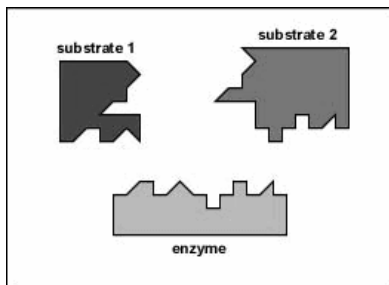
- In this form of catalysis, the induced structural rearrangements that take place with the binding of substrate and enzyme ultimately produce strained substrate bonds,
  - Which more easily attain the transition state.
- The new conformation often forces substrate atoms and bulky catalytic groups, such as aspartate and glutamate, into conformations that strain existing substrate bonds.

## Enzyme Substrate Interactions

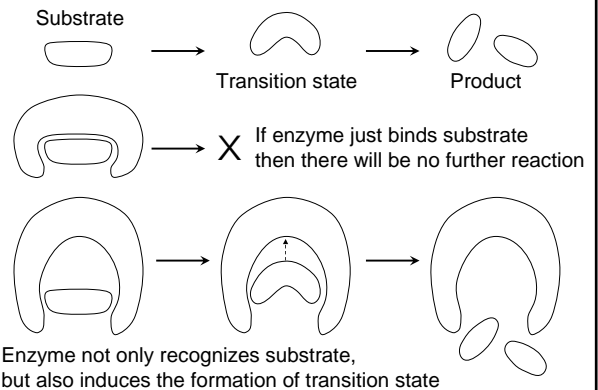
### Catalysis by Proximity and Orientation:

- Enzyme-substrate interactions orient reactive groups and bring them into proximity with one another.
  - In addition to inducing strain, groups such as aspartate are frequently chemically reactive as well, and their proximity and orientation toward the substrate thus favors their participation in catalysis.

## Enzyme Substrate Interactions



## Stickase

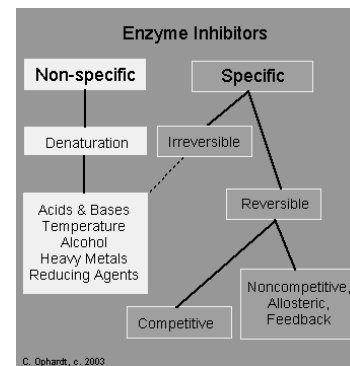


## Enzyme Inhibitors

### Enzyme Inhibitors

- Specific enzyme inhibitors regulate enzyme activity and help us understand mechanism of enzyme action. (Denaturing agents are not inhibitors)
- **Irreversible inhibitors** form covalent or very tight permanent bonds with aa at the active site of the enzyme and render it inactive. 3 classes: group specific reagents, substrate analogs, suicide inhibitors
- **Reversible inhibitors** form an EI complex that can be dissociated back to enzyme and free inhibitor. 3 groups based on their mechanism of action: competitive, non-competitive and uncompetitive.

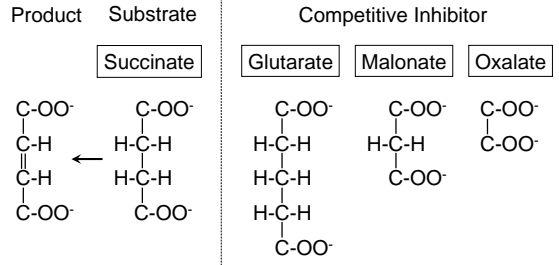
## Enzyme Inhibitors



### Competitive Inhibition

- Has a structure similar to substrate
- Occupies active site
- Competes with substrate for active site
- Has effect reversed by increasing substrate concentration

### Competitive Inhibition



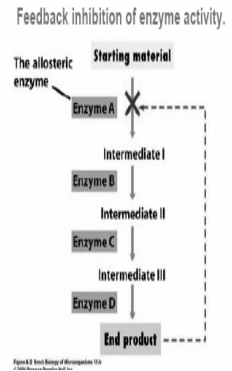
Succinate Dehydrogenase

### Non-Competitive Inhibition

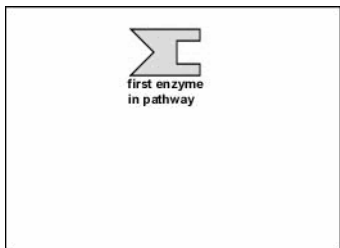
- Does not have a structure like substrate
- Binds to the enzyme but not active site
- Changes the shape of enzyme and active site
- Substrate cannot fit altered active site
- No reaction occurs
- Effect is not reversed by adding substrate

### Non-Competitive Inhibition

**Feedback Control**  
The activity of the **first enzyme** of the pathway is inhibited by the **end product**, thus controlling **production of end product**.



### Non-Competitive Inhibition



- When the end product (inhibitor) of a pathway combines with the allosteric site of the enzyme, this alters the enzyme's active site so it can no longer bind to the starting substrate of the pathway. This blocks production of the end product.

### Enzyme Inhibition (Mechanism)

	▶ Competitive	■ Non-competitive	■ Uncompetitive
Cartoon Guide			
Equation and Description	$E + S \rightleftharpoons ES \rightarrow E + P$ $+ I$ $\downarrow$ $EI$	$E + S \rightleftharpoons ES \rightarrow E + P$ $+ I$ $+ I$ $\downarrow \uparrow$ $EI + S \rightarrow EIS$	$E + S \rightleftharpoons ES \rightarrow E + P$ $+ I$ $\downarrow \uparrow$ $EIS$
Equation and Description	[I] binds to free [E] only, and competes with [S]; increasing [S] overcomes inhibition by [I].	[I] binds to free [E] or [ES] complex; increasing [S] can not overcome [I] inhibition.	[I] binds to [ES] complex only, increasing [S] favors the inhibition by [I].