

## INTRODUCTION TO ENZYMES

- Enzymes are usually proteins (some RNA)
- In general, names end with suffix “ase”
- Enzymes are catalysts
  - increase the rate of a reaction
  - not consumed by the reaction
  - act repeatedly to increase the rate of reactions
  - Enzymes are often very “specific” – promote only 1 particular reaction
  - Reactants also called “substrates” of enzyme

<u>catalyst</u>	<u>rate enhancement</u>
non-enzymatic (Pd)	$10^2$ - $10^4$ fold
enzymatic	up to $10^{20}$ fold

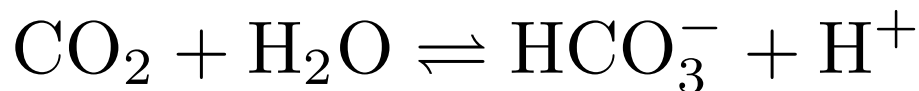
- How much is  $10^{20}$  fold?

<u>catalyst</u>	<u>time for reaction</u>
yes	1 second
no	$3 \times 10^{12}$ years

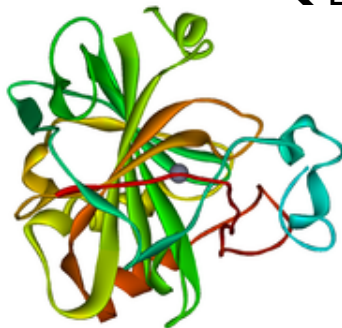
- $3 \times 10^{12}$  years is 500 times the age of the earth!

## Carbonic Anhydrase

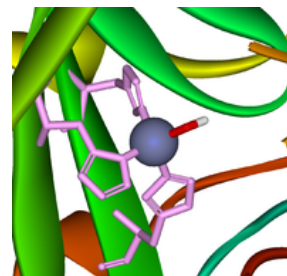
Tissues →



← Lungs and Kidney



$10^7$  rate  
enhancement



Facilitates the transfer of carbon dioxide from tissues to blood and from blood to alveolar air

Many enzyme names end in –ase

**TABLE 6-1 | Rate Enhancements of Enzymes**

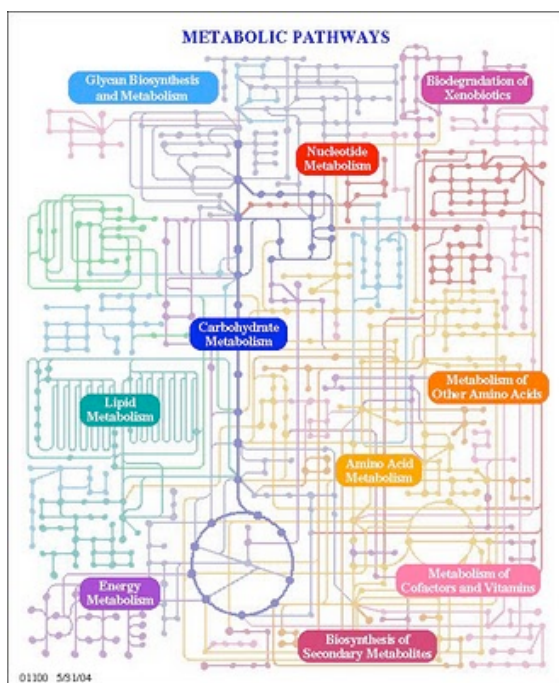
Enzyme	Half-Time (uncatalyzed) <sup>a</sup>	Uncatalyzed Rate (s <sup>-1</sup> )	Catalyzed Rate (s <sup>-1</sup> )	Rate Enhancement (catalyzed rate/uncatalyzed rate)
Orotidine-5'-monophosphate decarboxylase	78,000,000 years	$2.8 \times 10^{-16}$	39	$1.4 \times 10^{17}$
Staphylococcal nuclease	130,000 years	$1.7 \times 10^{-13}$	95	$5.6 \times 10^{14}$
Adenosine deaminase	120 years	$1.8 \times 10^{-10}$	370	$2.1 \times 10^{12}$
Chymotrypsin	20 years	$1.0 \times 10^{-9}$	190	$1.7 \times 10^{11}$
Triose phosphate isomerase	1.9 years	$4.3 \times 10^{-6}$	4,300	$1.0 \times 10^9$
Chorismate mutase	7.4 hours	$2.6 \times 10^{-5}$	50	$1.9 \times 10^6$
Carbonic anhydrase	5 seconds	$1.3 \times 10^{-1}$	1,000,000	$7.7 \times 10^6$

<sup>a</sup>The half-times of very slow reactions were estimated by extrapolating from measurements made at very high temperatures.

[Data mostly from Radzicka, R., and Wolfenden, R., *Science* 267, 90–93 (1995).]

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## Why Enzymes?



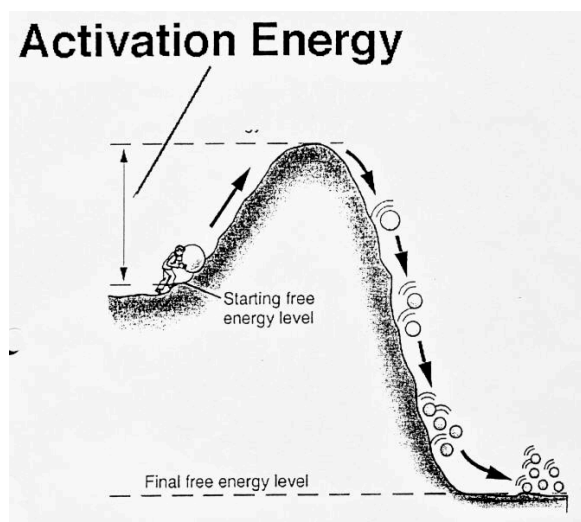
- Accelerate and control the rates of vitally important biochemical reactions
- Greater reaction specificity
- Milder reaction conditions
- Capacity for regulation
- Enzymes are the agents of metabolic function.
- Metabolites have many potential pathways
- Enzymes make the desired one most favorable

- **Enzymes are necessary for life to exist – otherwise reactions would occur too slowly for a metabolizing organism**
- **Enzymes DO NOT change the equilibrium constant of a reaction** (accelerates the rates of the forward and reverse reactions equally)
- **Enzymes DO NOT alter the standard free energy change, ( $\Delta G^\circ$ ) of a reaction**
  1.  $\Delta G^\circ$  = amount of energy consumed or liberated in the reaction
  2. Quantity that determines if a reaction is energetically favorable.

3. Reaction is thermodynamically favorable or SPONTANEOUS if  $\Delta G^\circ$  is **negative**.  
(Note: Spontaneous does NOT mean instantaneous; energy must be supplied to START a reaction which then proceeds with a release of energy)
  4. i.e. **Enzymes DO NOT change thermodynamics** (can't make a reaction spontaneous)
- **Enzymes DO decrease the activation energy of a reaction ( $\Delta G^\ddagger$ )**
    1. Activation Energy is the energy required to start a reaction.
    2. Related to **rate**: Less energy needed to start the reaction, the faster it'll go
    3. Enzymes DO increase the rate of reactions that are otherwise possible by **DECREASING** the activation energy of a reaction
    4. Lowering the  $E_a$  increases the rate constant,  $k$ , and thereby increases the rate of the reaction

### Activation Energy Diagrams:

Supply energy by pushing a rock up a hill so that it can slide down the other side, releasing energy.



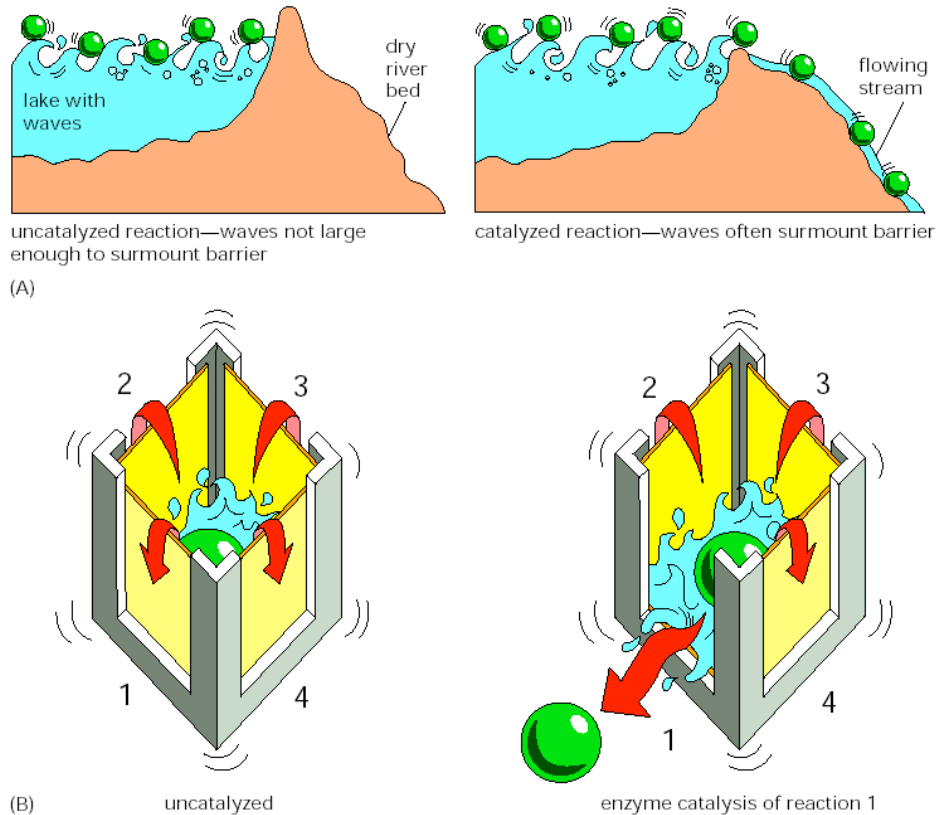
Think of activation energy as the BARRIER required to make a product.

Most stable product is the one with the lowest energy.

Most reactions require a “push” to get them started! “Push” is called “energy of activation” for reaction - Also represented by  $E_A$

## Floating Ball Analogies for Enzyme Catalysis

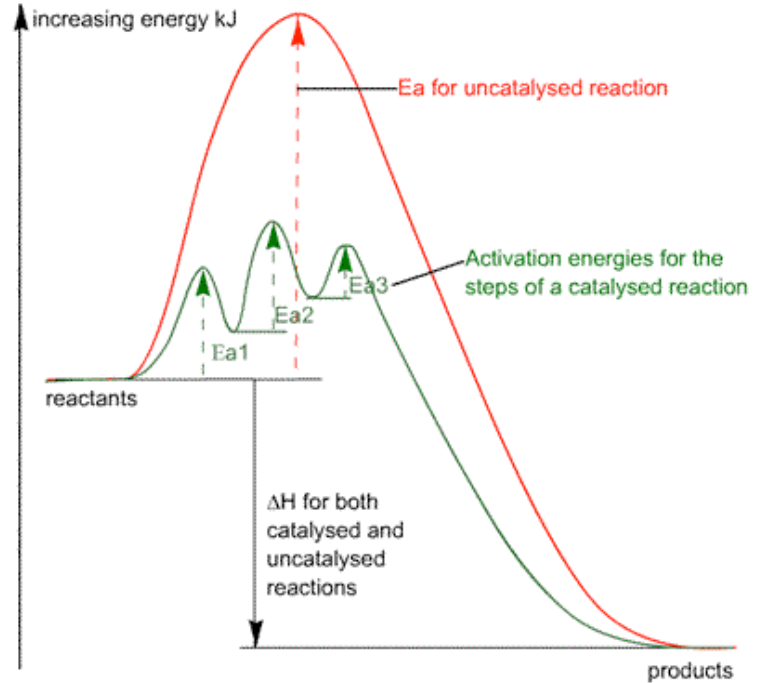
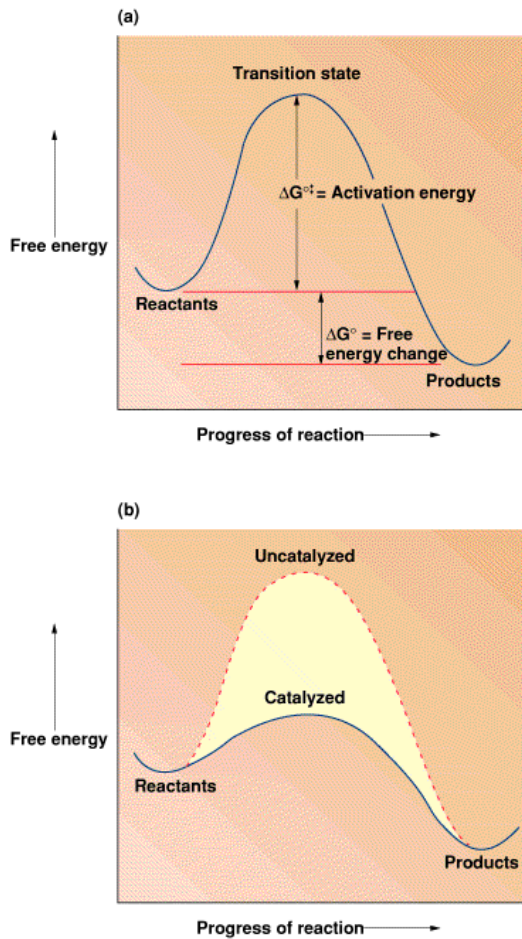
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### Legend:

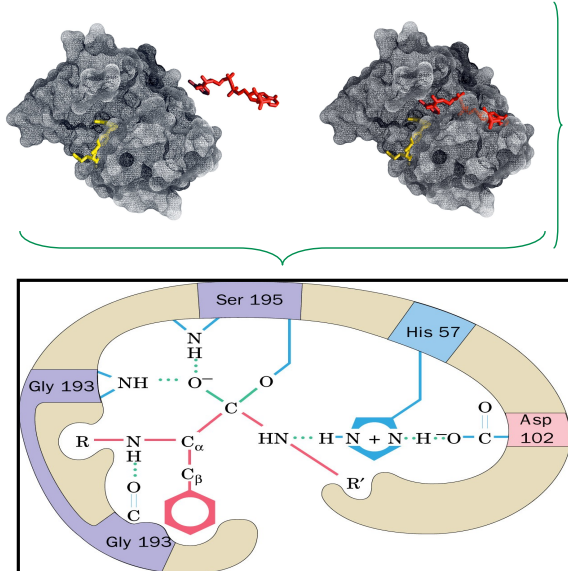
(A) A barrier dam is lowered to represent enzyme catalysis. The green ball represents a potential enzyme substrate (compound X) that is bouncing up and down in energy level due to constant encounters with waves (an analogy for the thermal bombardment of the substrate with the surrounding water molecules). When the barrier (activation energy) is lowered significantly, it allows the energetically favorable movement of the ball (the substrate) downhill. (B) The four walls of the box represent the activation energy barriers for four different chemical reactions that are all energetically favorable, in the sense that the products are at lower energy levels than the substrates. In the left-hand box, none of these reactions occurs because even the largest waves are not large enough to surmount any of the energy barriers. In the right-hand box, enzyme catalysis lowers the activation energy for reaction number 1 only; now the jostling of the waves allows passage of the molecule over this energy barrier only, inducing reaction 1.

For a chemical or enzymatic reaction, it is no different!



- A catalyst lowers  $E_a$  by providing a *different mechanism & different pathway*, for the reaction through a new, lower energy pathway.

**Weak Interactions Between Enzyme and Substrate are Optimized in the Transition State**



Non-Covalent Interactions!

**Binding energy** ( $\Delta G_B$ )--- free energy released in forming multiple weak bonds and interactions between an enzyme and its substrate

Ensures favorable formation of the ES complex, but not too favorable

**Binding energy is used to lower the activation energy barrier**

**Transition State:**

- Old bonds break and new ones form.
- Substance is neither substrate nor product
- Unstable short lived species with an equal probability of going forward or backward.
- Strained intermediate

- See visually how  $\Delta G^\circ$  would be **negative**  $\rightarrow$  **overall DECREASE in potential free energy = SPONTANEOUS! Not instantaneous...**

**ENZYMES work by LOWERING the activation energy:** a greater fraction of molecules can cross the lower barrier and react to form product – just like ball on waves.

**In the diagram:**

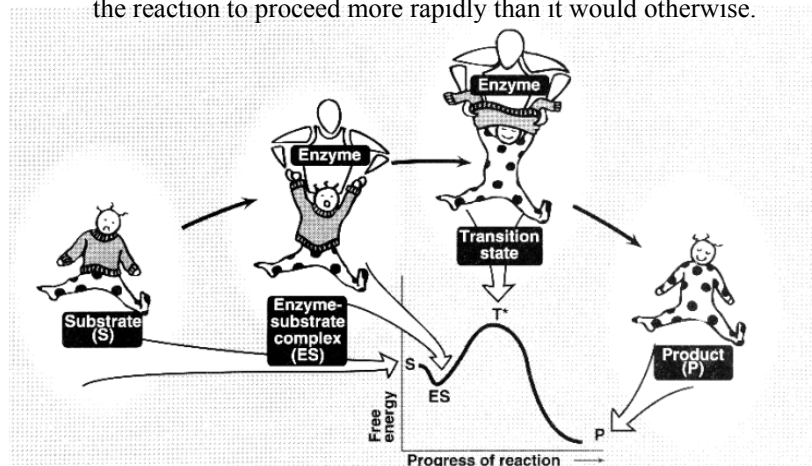
- Lowers the energy of the transition state and activation energy (both forward and reverse!)
- Increases the rate – since less energy is needed to start the reaction, the faster it will proceed.
- Increases the rate of both forward and reverse reactions.
- Enzyme does NOT change the energy of the reactants (substrates) or products -  $\Delta G^\circ$  constant
- Free Energy Value for products is lower that value for reactants (substrates) – Reaction is thermodynamically favorable but needs a push!

**How does an enzyme lower the activation energy?**

Often by holding reactant molecules in a position where they react more readily. Shape of enzyme allows reactants to fit into a specific place – called the “**active site**” of an enzyme  
Uses energy gained by binding substrate. (More later.....)

## Catalysis

Enzymes poise all the reactants in a arrangement that allows the reaction to proceed more rapidly than it would otherwise.



## ENZYMES II

- Enzymes physically interact with their substrates to effect catalysis



where...

E = enzyme

ES = enzyme/substrate complex

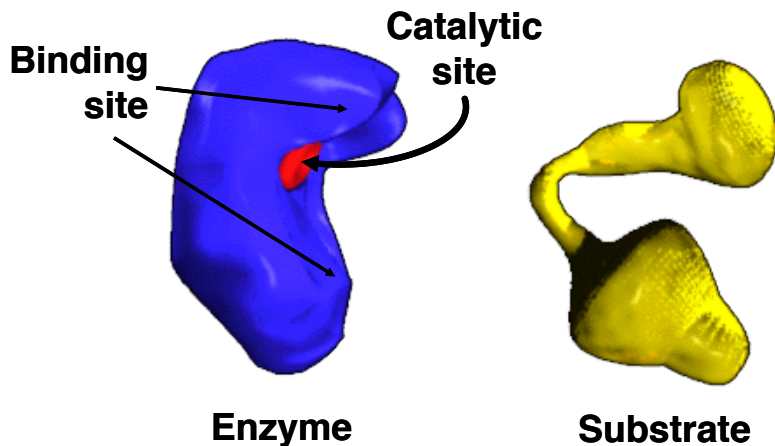
ES\* = enzyme/transition state complex

EP = enzyme/product complex

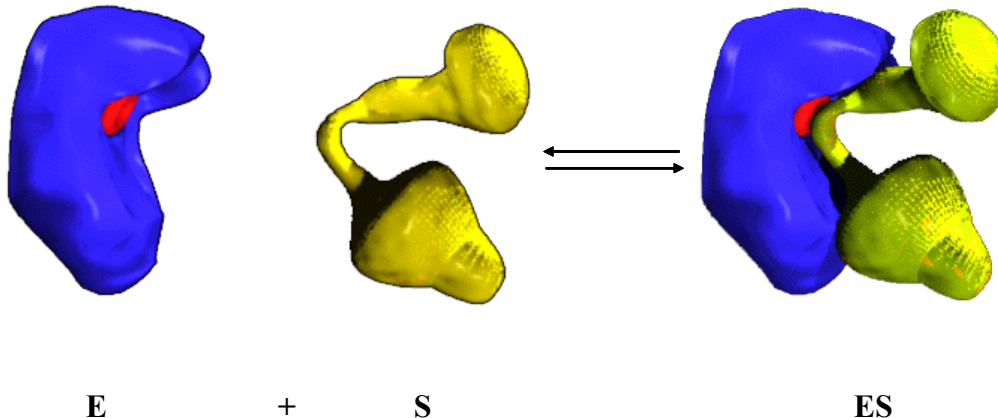
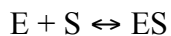
P = product

- Substrates bind to the enzyme's **active site**
  - pocket in the enzyme
  - Binding site** = where substrate binds; area that holds substrate in place via non-covalent interactions
  - Catalytic site** = where reaction takes place

### THE PLAYERS:

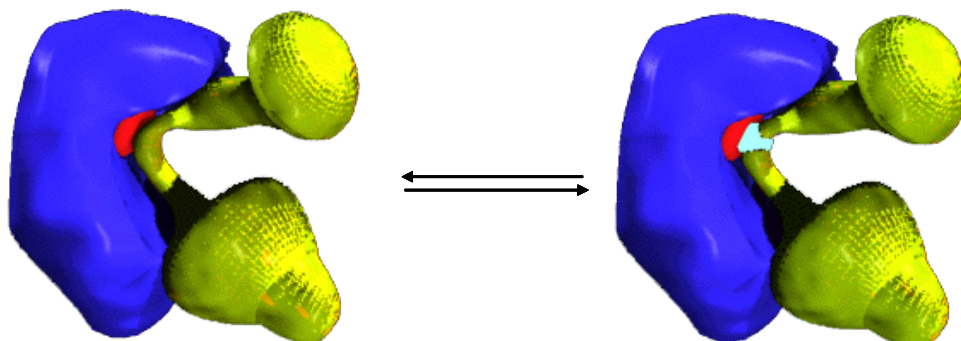


**First Step:** Enzyme binds to substrate molecule to form an enzyme – substrate complex



**Second Step:** Formation of the transition state complex where the bound substance is neither product nor reactant -  $ES \leftrightarrow ES^*$

**Note Change!!**  $ES \neq ES^*$

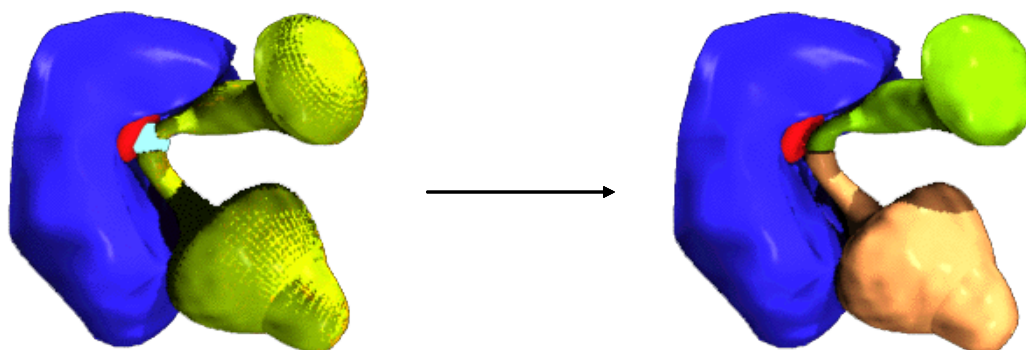


ES

ES\*

**Third Step:** Formation of the enzyme – product complex  $ES^* \rightarrow EP$

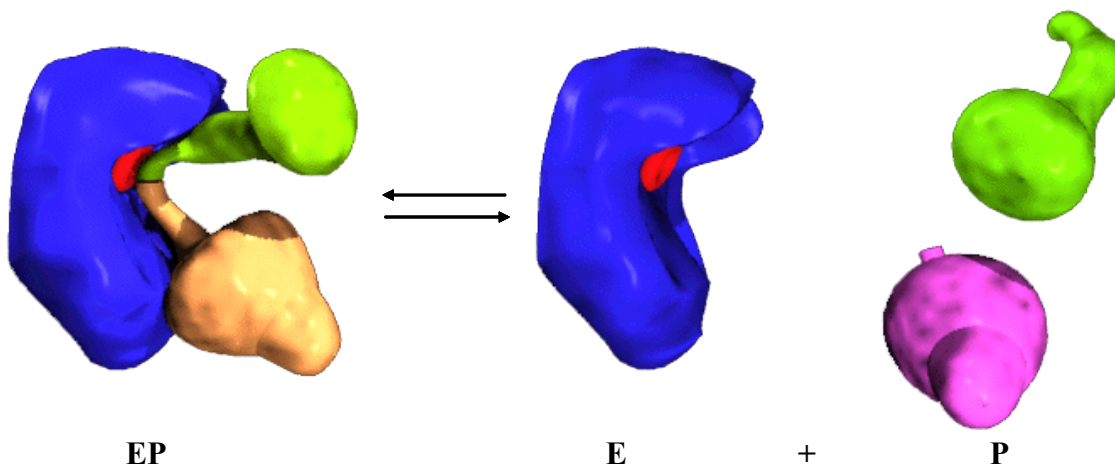
ES\* → EP



ES\*

EP

**Fourth Step:** Release of product  $EP \leftrightarrow E + P$



EP

E

+

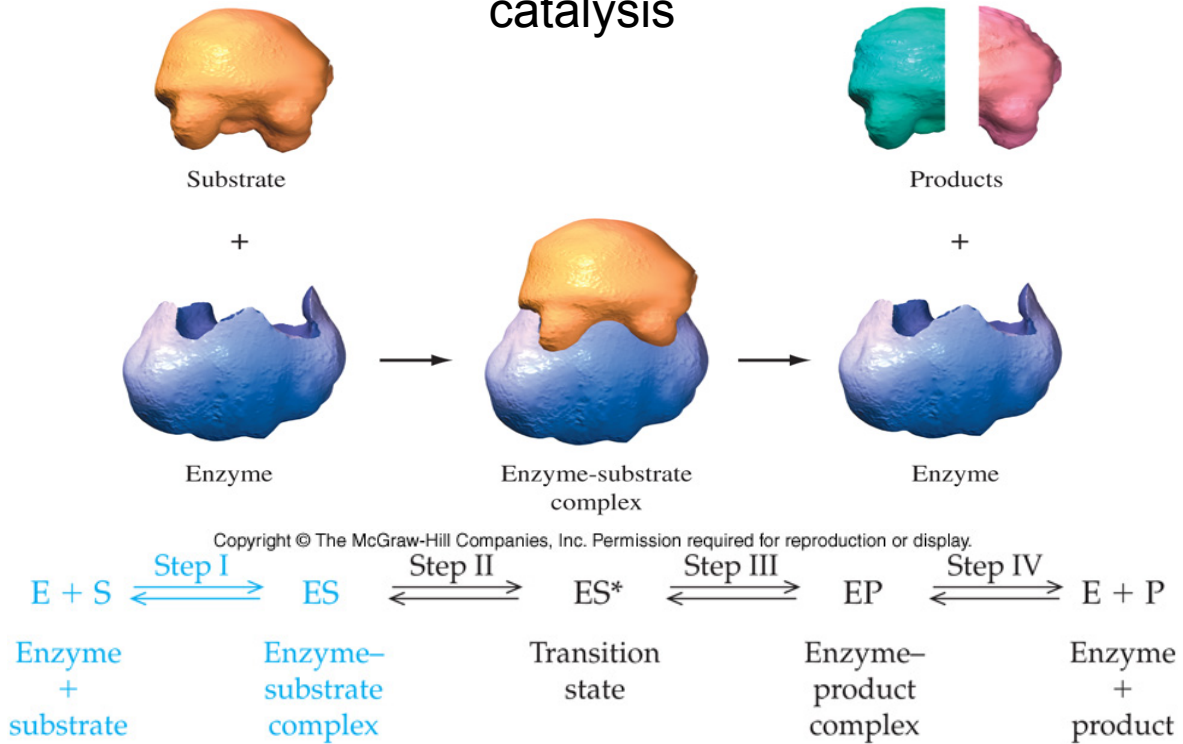
P

**Once product is released, enzyme is unchanged and can carry out another reaction – only one at a time!**



# Enzyme Catalysis

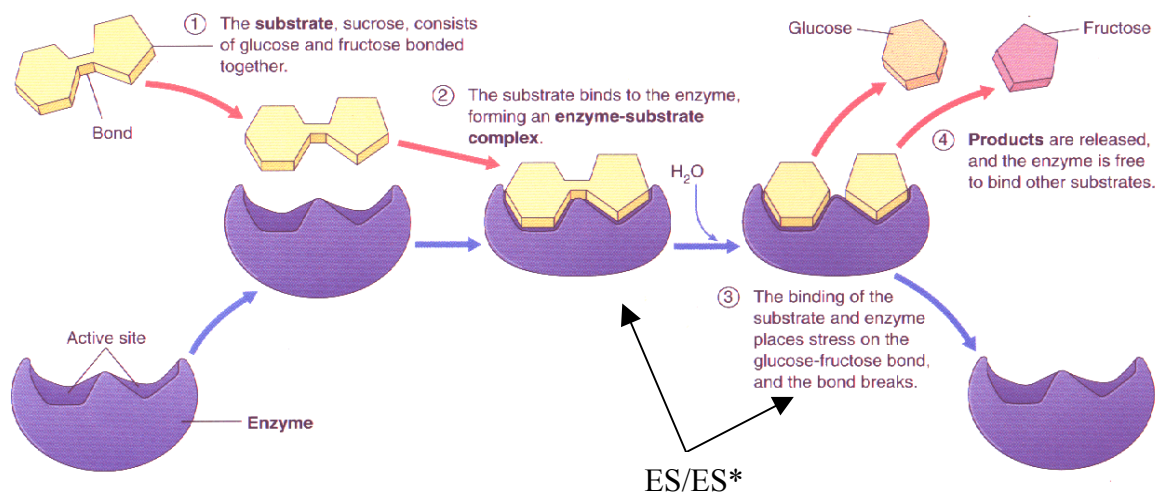
**Enzymes** are proteins that provide a 3D surface for catalysis



## Enzymatic cleavage of Sucrose:

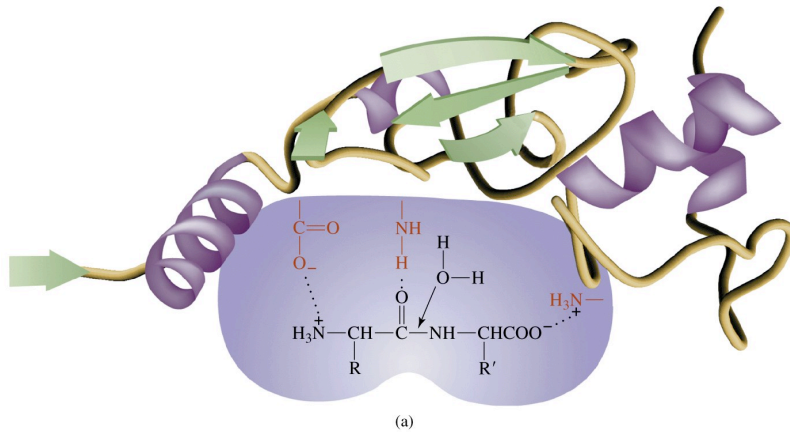
Once product is released, enzyme is available to accept another substrate molecule.

Enzyme can only work on one substrate molecule at a time and is NOT changed during the reaction.

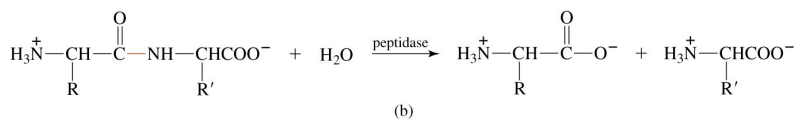


### ENZYMATIC CLEAVAGE OF SUCROSE

## FIRST STEP: SUBSTRATE BINDING



**BINDING OF A DIPEPTIDE TO A HYPOTHETICAL ACTIVE SITE**



**Dipeptide cleaved into 2 aa**

Figure 5-8 Concepts in Biochemistry, 3/e  
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**ACTIVE SITE:** Pocket in the enzyme where substrates bind and catalytic reaction occurs

- **Has SPECIFICITY – can discriminate among possible substrate molecules**
  1. Others recognize a functional group (Group specificity)
  2. Some only recognize one type of molecule (e.g. D vs L isomers) (Absolute specificity)
- **Active site is a relatively small 3-D region within the enzyme**
  1. As we saw, typically a small cleft or crevice on a large protein
- **Substrates bind in active site by weak non-covalent interactions**
  - A. hydrogen bonding
  - B. hydrophobic interactions
  - C. ionic interactions
- **The interactions hold the substrate in the proper orientation for most effective catalysis**
- **The ENERGY derived from these interactions = “BINDING ENERGY”**
- **Using this energy, enzymes both lower the activation energy and stabilize the transition state complex (ES<sup>\*</sup>). Each non-covalent interaction provides energy to stabilize the transition state.**

**TWO MODELS FOR ENZYME/SUBSTRATE INTERACTIONS:****1. Lock and Key Model:**

- A. Substrate (key) fits into a perfectly shaped space in the enzyme (lock)
- B. As we've said, there is lots of similarity between the shape of the enzyme and the shape of the substrate
- C. Highly stereospecific
- D. Implies a very RIGID inflexible active site
- E. Site is preformed and rigid

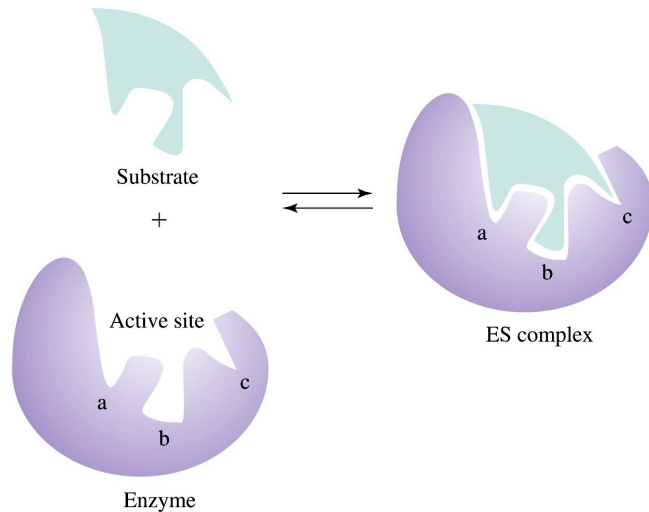


Figure 5-9 Concepts in Biochemistry, 3/e  
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**2. Induced Fit Model (Hand in Glove analogy)**

- A. Takes into account the flexibility of proteins
- B. A substrate fits into a general **shape** in the enzyme, causing the enzyme to change shape (conformation); close but not perfect fit of E + S
- C. Change in protein configuration leads to a near perfect fit of substrate with enzyme

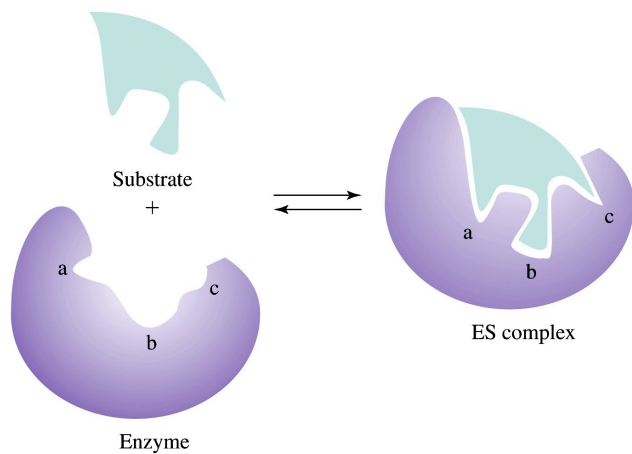
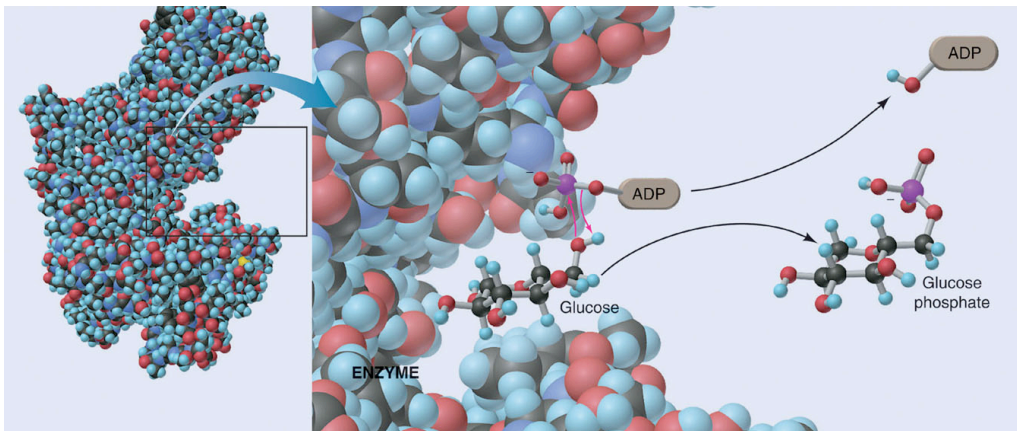
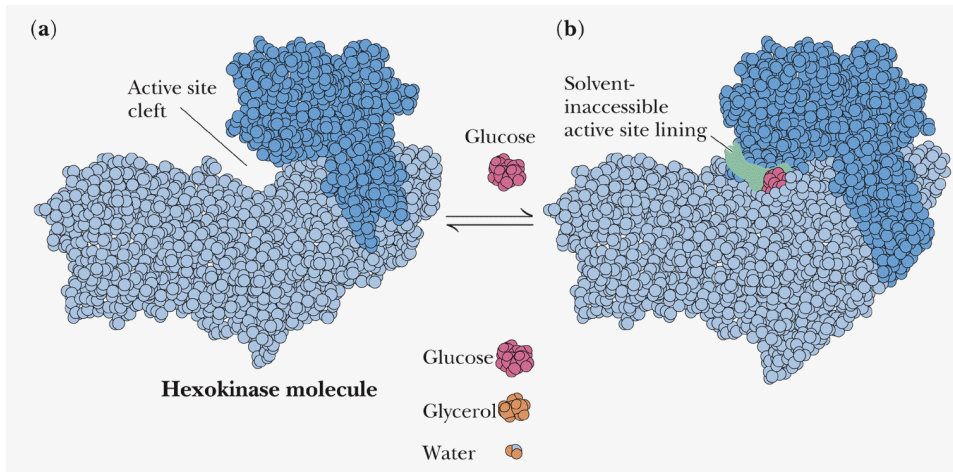
**Induced Fit Model**

Figure 5-10 Concepts in Biochemistry, 3/e  
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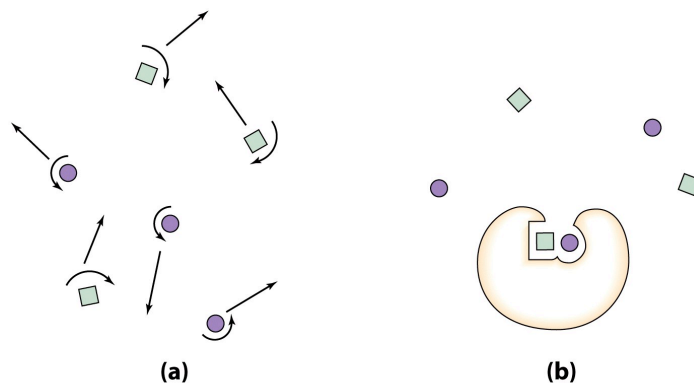
**Glucose binding to hexokinase: Note Conformational Change in Enzyme**



D. When substrate is completely held in active site, it takes on characteristics of the **TRANSITION STATE** for the reaction

(Non-covalent interactions between the enzyme and substrate change the 3-D structure of the active site, conforming the shape of the active site to the shape of the substrate in its transition state conformation ( $E + S \leftrightarrow ES^*$ )

- i. Enzyme puts substrate in correct **ORIENTATION** to make the reaction proceed
- ii. Enzyme substrate in close **PROXIMITY** to groups on the enzyme necessary for catalysis—
  1. Catalysis is begun by amino acids making up the active site of the enzyme; R groups are usually crucial in catalysis.

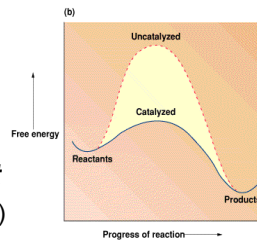


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## Enzymes Lower the Activation Energy of the Reaction

How does an enzyme lower  $E_a$ ?

*By Stabilizing the Transition State!*



- Puts molecules in *close proximity* to react
  - increases the local concentration of reactants)
- Puts molecules in *correct orientation*
  - Reactants are not only near each other on enzyme, they're oriented in optimal position to react, making it possible to always collide in the **correct orientation**.

### Proximity & Orientation

iii. Called “transition state theory”

1. Enhances the formation of and stabilize the highly energetic transition state
  2. Transition state binds more tightly than substrate or product
- **Enzyme binds tightly to the transition state species (i.e. substrates that have been strained toward the structures of the product!**
    - i. Binding energy helps reach and stabilize the transition state → lowers activation energy → increases rate!
  - **Transition state stabilization accomplished through close complementarity in shape and charge between the active site and the transition state.**

Reaction proceeds – bonds are broken and new ones formed, transforming  $S \rightarrow P$

Following catalysis, the product(s) no longer fits the active site and is released

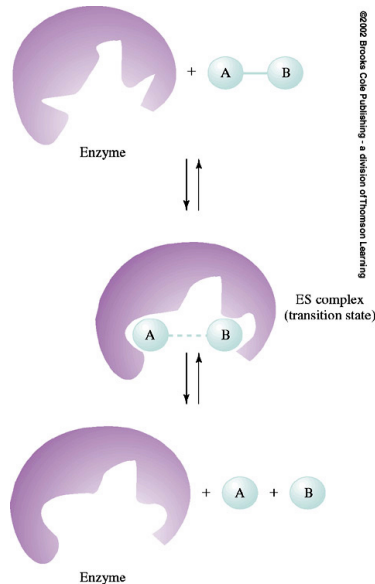
- A. Many drugs that act as inhibitors of an enzyme are designed to resemble the substrate transition state.

## Transition-State Analog Enzyme Model

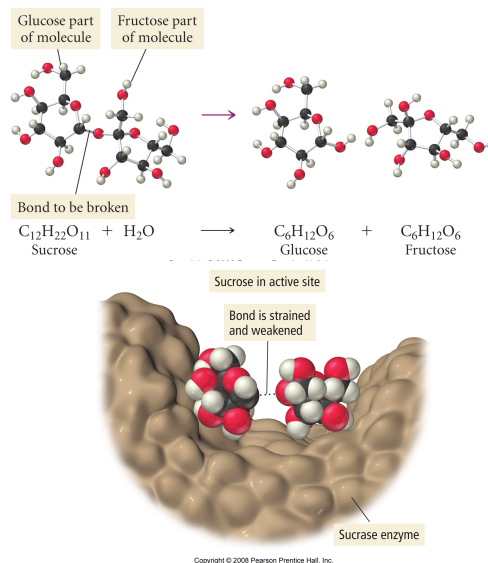
The binding of the substrate results in the distortion of the **substrate** and **enzyme** in ways that makes the chemical reaction easier.

*Enzyme active sites are complementary not to the substrate per se, but to the transition state through which substrates pass as they are converted into products during an enzymatic reaction*

Enzymes bind the transition state structure more tightly than the substrate



## Enzymatic Hydrolysis of Sucrose



Also see <http://www.boyerbiochem.com> – **Interactive Animations: Enzyme Specificity**

### Induced Fit Model and Enzyme Videos:

<http://www.youtube.com/watch?v=z8lG8X9ZvxQ&feature=Playlist&p=399E40B58925C7BF&index=0&playnext=1>

<http://www.5min.com/Video/Enzyme-Action-The-Induced-Fit-Model-150616169>