بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

BioChemistry

وَلَقَدْ خَلَقْنَا الْإِنسَانَ وَنَعْلَمُ مَا تُوَسْوِسُ بِهِ نَفْسُهُ ۖ وَنَحْنُ أَقْرَبُ إِلَيْهِ مِنْ حَبْلِ الْوَرِيدِ

Final | Lecture #11

Enzymes pt.1

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Hamza Saraireh

Reviewed by : Yaman Khalil

Color coding used in the modified:

- *Black: the original slides*
- *Blue: the doctor’s explanation/words*
- *Gray: additional information and explanation*
- *Red: important information*

Enzymes I

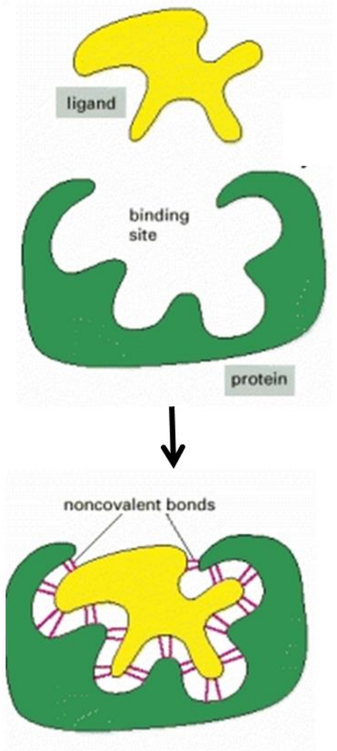
Characteristics and classification

Prof. Mamoun Ahram

General properties of proteins

Proteins can't function on their own they have to have interactions, usually those interactions are through something called a ligand

- The function of proteins depends on their ability to bind other molecules (ligands).



Usually a smaller molecule binds to a larger one

- Ligand: a substance that forms a complex with a biomolecule, **usually via non-covalent** interactions, to serve a biological purpose.

- Two properties of a protein characterize its interaction with ligands:

Dictate/ determine

  - Affinity: the strength of binding between a protein and other molecules.

How strongly does X binds to protein A

  - Specificity: the ability of a protein to bind one molecule in preference to other molecules.

It means that a protein can bind to this ligand but not to another one

when talking about selectivity, you are considering how strong the bond between ligand X and protein A relative to ligand Y
Meaning protein A can bind to X or Y but it can bind to X more strongly

What are enzymes?

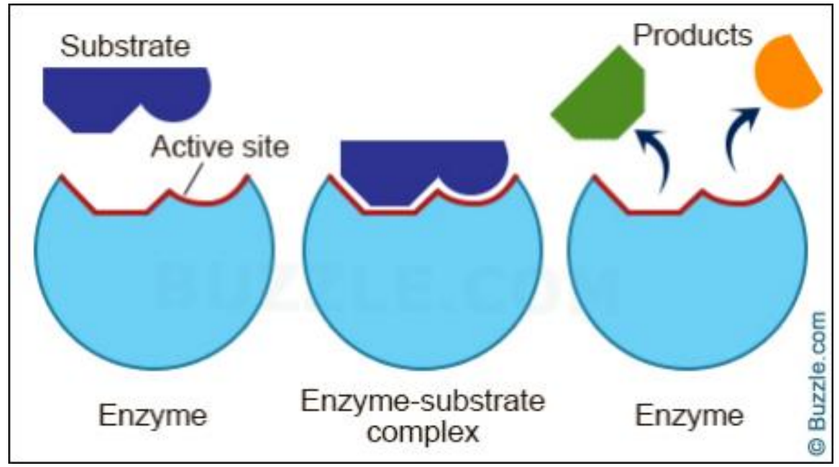
- Enzymes: Specialized **proteins** that accelerate (**catalyze**) chemical reactions under biological conditions.
  - Exception: ribozymes (*to be discussed*)
  - In enzymatic reactions, reactants are known as substrates.

RNA molecule who can act as an Enzyme.

- Most enzymes have very specific functions in converting specific substrates to products.
- Enzymes are catalysts.
  - They exist in small amounts relative to the reactants.
  - They increase the rate of a reaction.
  - At the completion of the reaction, **they revert to their original structure.**

One enzyme can make millions of reactions so u literally need very small amount of it.

If the structure of the enzyme stays changed after the reaction, it will be no longer an enzyme (it will be a reactant or substrate).



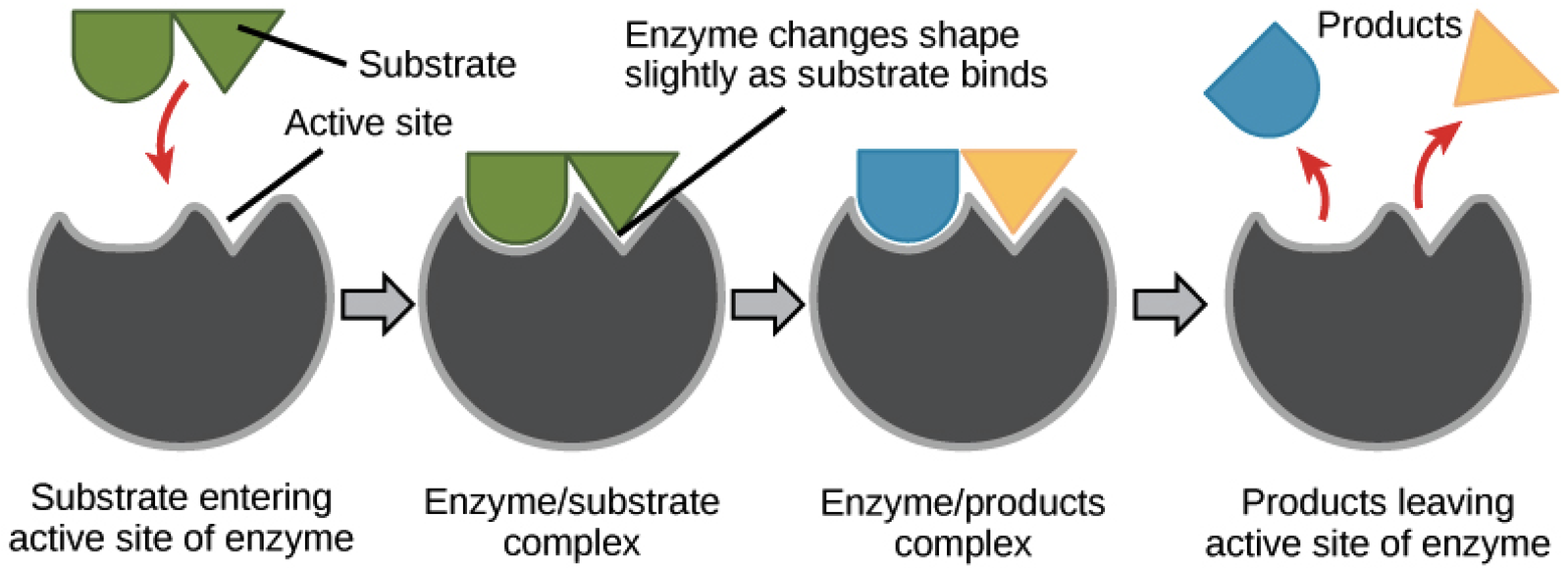
How can we express an enzymatic reaction?

In reality this complex doesn't exist, usually the substrate is converted to a product and the product is released right away

- Simple expression of enzymatic reaction:

$E + S \leftrightarrows ES \leftrightarrows EP \leftrightarrows E + P$ For further simplicity: $E + S \leftrightarrows ES \leftrightarrows E + P$

E = free enzyme; S = free substrate, ES = enzyme-substrate complex; P = product of the reaction; and EP = enzyme-product complex before the product is released



What do enzymes do?

- Enzymes accelerate reactions (usually within a range of 10^6 to 10^{14}).
  - Example: Catalase (10^8) & carbonic anhydrase (10^6)

Do not memorize the numbers

  - Carbonic anhydrase:

$$CO_2 + H_2O \xleftrightarrow{\text{Carbonic anhydrase}} H_2CO_3$$

    - One enzyme molecule hydrates 10^6 molecules of CO_2 per second (versus 1 per 10 seconds for uncatalyzed reactions)

  - Catalase:

$$2\,H_2O_2 \xrightleftharpoons{\text{Catalase}} 2\,H_2O + O_2\,(g)$$

Reaction Conditions	Relative Rate
No catalyst	1
Platinum surface	2.77×10^4
Catalase	6.51×10^8

Do not memorize

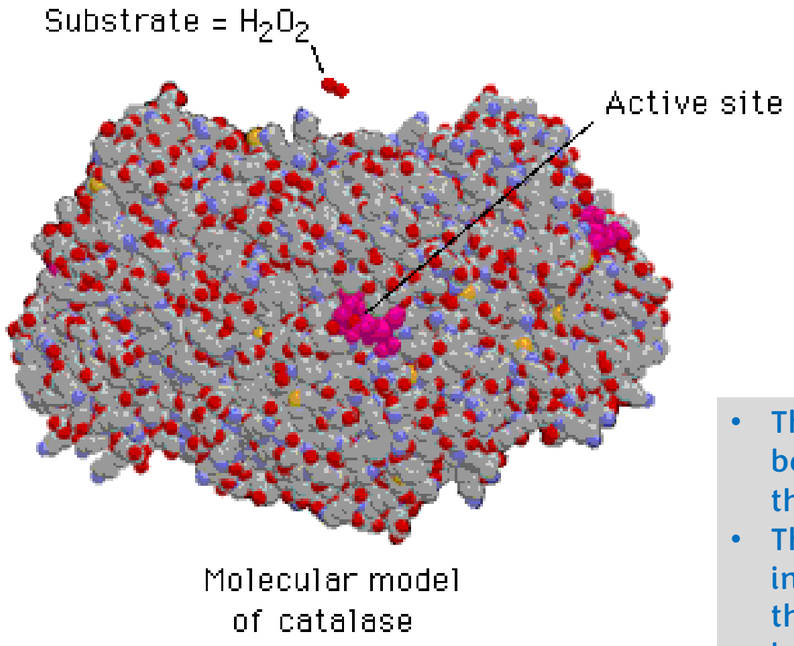
The surface can accelerate the reaction we will learn about the mechanism in the following lectures

Where does the reaction occur?

The interactions between the substrate and the enzyme are non-covalent interactions

- Each enzyme has a specific three-dimensional shape called **the active site** (a **region where the biochemical reaction occurs**).

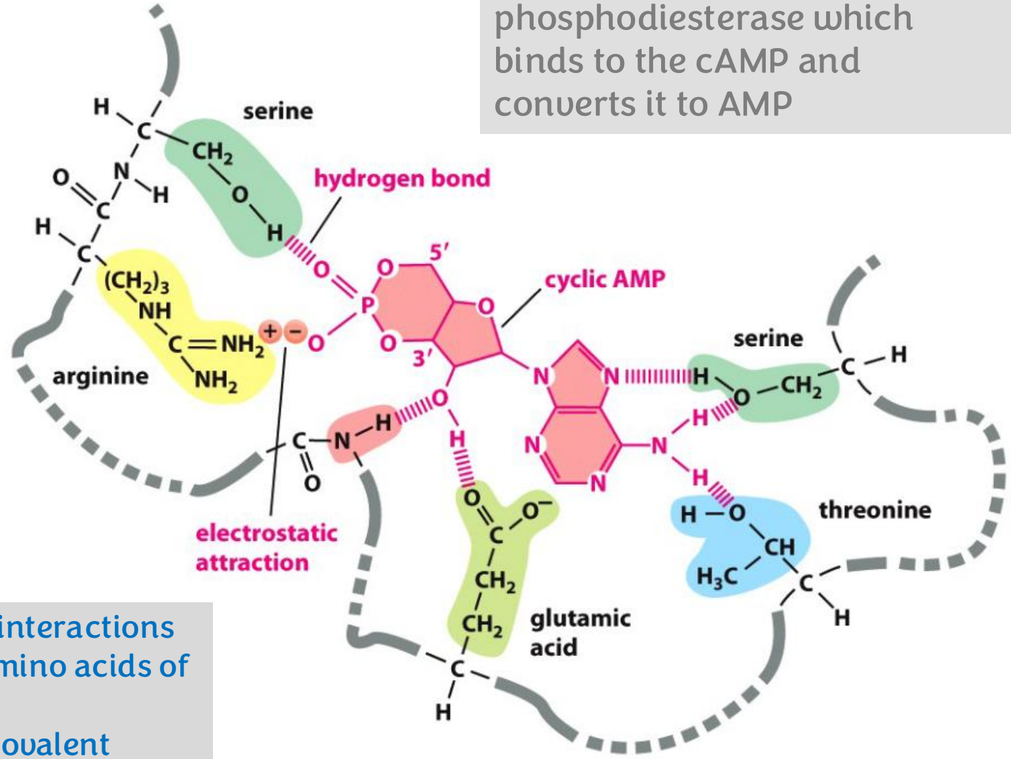
Active sites are small portions of the enzyme they are composed of specific amino acids



- There are specific non-covalent interactions between the substrate and the amino acids of the active site (specificity)
- The numbers and types of non-covalent interactions determine the affinity (the more the electrostatic interactions and hydrogen bonds compared to van der waals, the more the affinity)

- The active site contains a specialized amino acid sequence that facilitates the reaction.

This enzyme is CAMP phosphodiesterase which binds to the cAMP and converts it to AMP

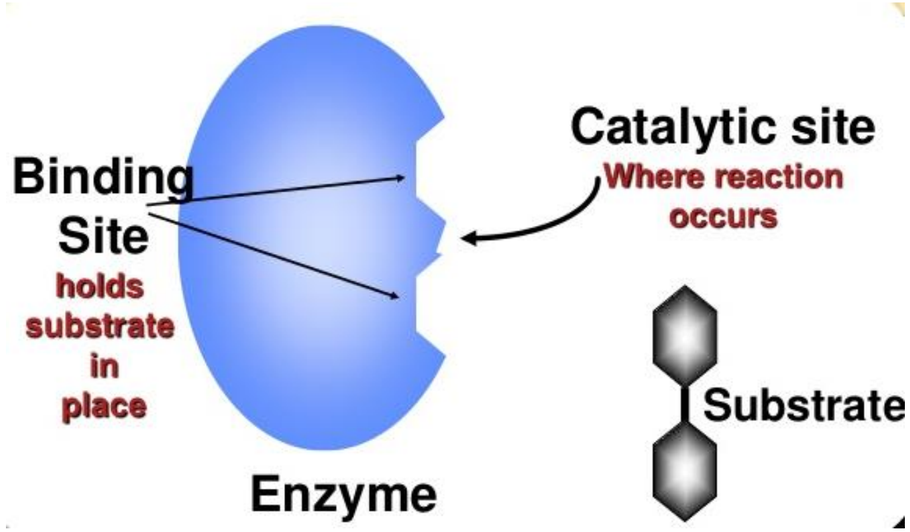


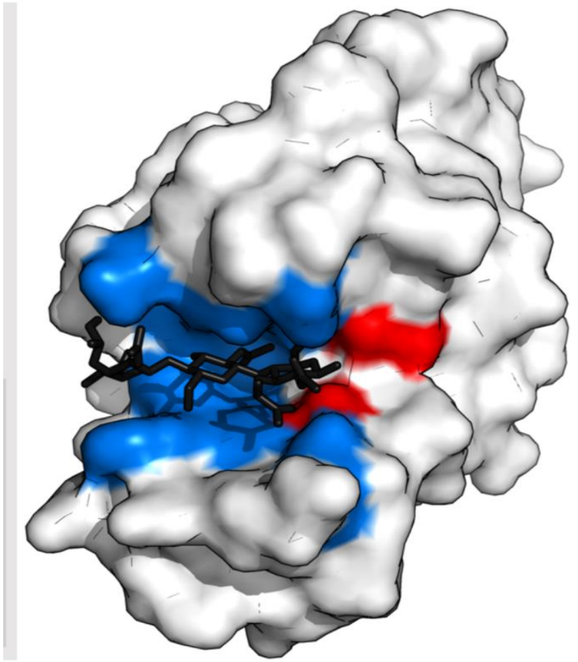
Catalytic and binding groups

- Within the active site are **two sub-sites**: the binding and catalytic sites.
- The catalytic site contains amino acid residues (catalytic group) that carry out the actual reaction. Catalytic site catalyzes the reactions
- **In some enzymes, the binding and catalytic sites are the same.**

It is like when u catch someone with one hand (binding site) and u punch him with another (catalytic site)

Both binding and catalytic sites determine the specificity





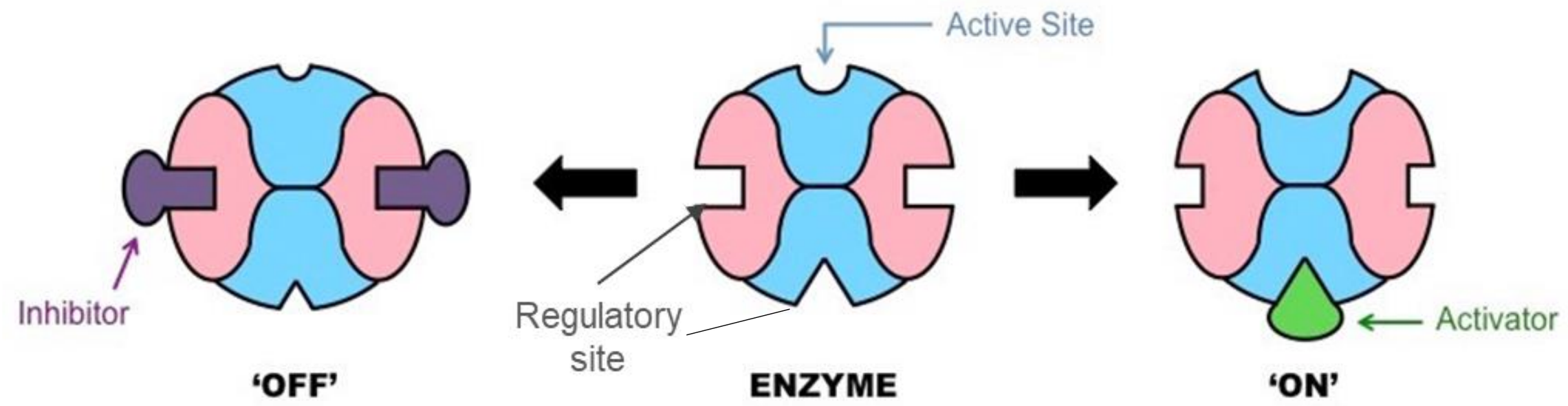
ACTIVE SITE

BINDING SITES	CATALYTIC SITE
Bind and orient substrate(s)	Reduce chemical activation energy

A regulatory site(s) can also be present.

- Binding of a substrate into the active site can be regulated by a *regulatory site* that is bound by a regulator (activator or inhibitor) of **either the catalytic activity of the enzyme or the binding of the substrate.**

Some enzymes have a regulatory site (another site in addition to the active site)



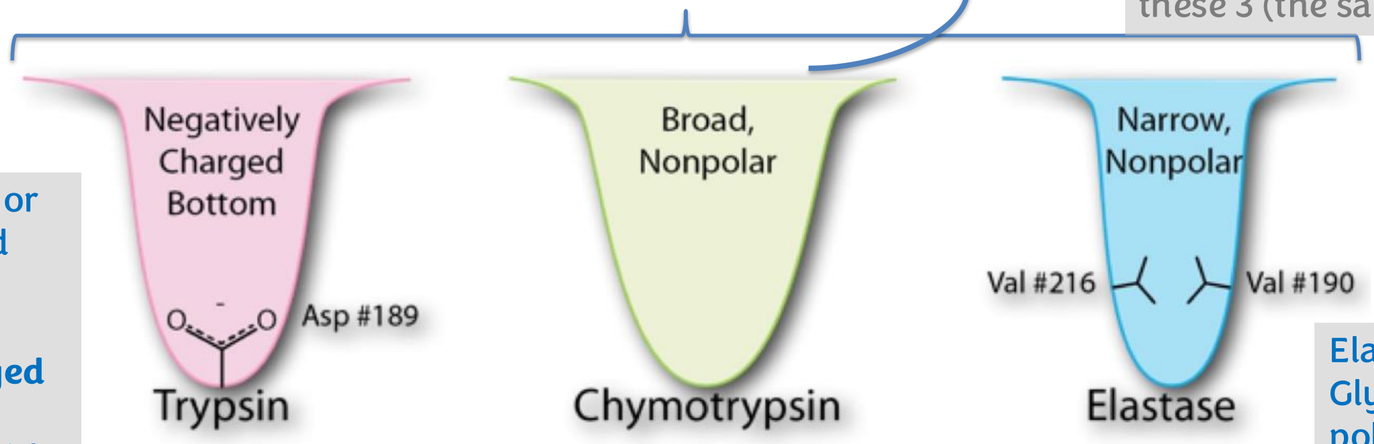
Binding specificity

The active site should fit the substrate in terms of shape and composition

- **The specificity of enzymes is due to their precise interaction of active sites with their substrates.**

Proteases: enzymes that cleaves proteins by breaking peptide bonds

Chymotrypsin recognises and cleaves of amino acids that are aromatic (Phe, Trp, Tyr), so the active site is expected to be **wide** and containing **non polar** amino acids (it cuts the chain after these 3 (the same mechanism of trypsin))



Trypsin binds to either Arg or Lys (positively charged and aliphatic AAs) So the enzyme's active site must contain a **negatively charged** Amino Acid (Asp it makes electrostatic interactions with Lys and Arg) and it -the active site- has to be **tall** for the molecule to enter(aliphatic) And this enzyme will cut the chain at the peptide bond directly after Arg or Lys

Lysine
Arginine

Phenylalanine
Tryptophan
Tyrosine

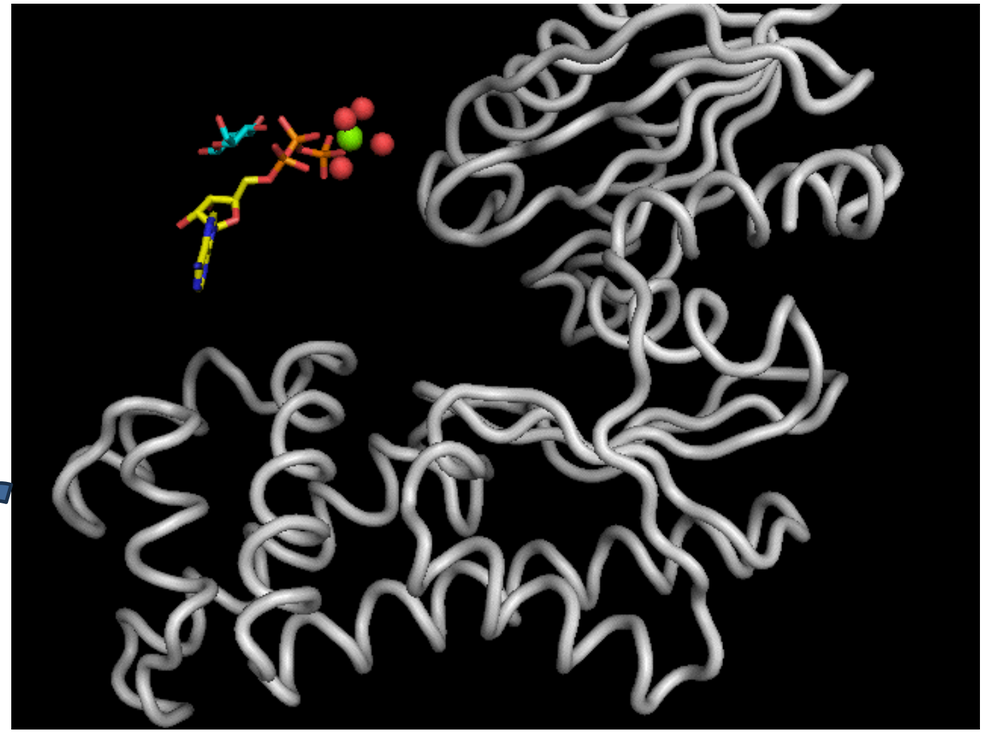
Glycine
Alanine
Valine

Elastase (works on elastin) recognises Gly, Ala, and Val which are small non polar amino acids, so the active site is expected to be non polar and narrow (these amino acids are small) (the same mechanism of the previous two)

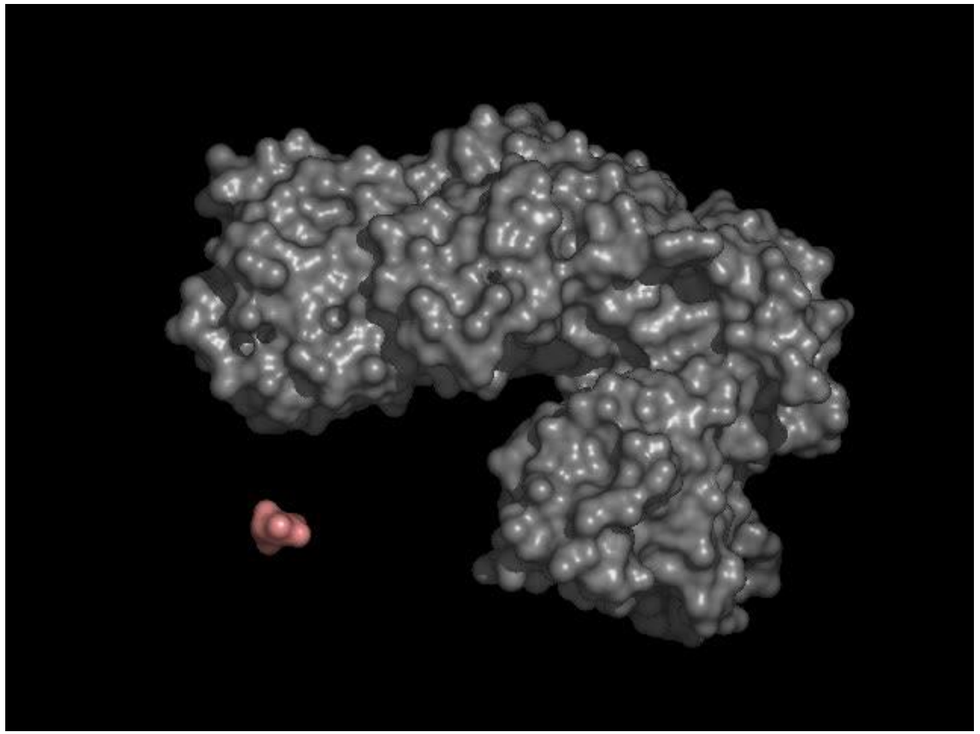
Features of active site 1

- It is **internal relative to the enzyme** and looks like a canal

Some amino acids on the surface in addition to the ones of the canal allows the substrate only to enter and prevents other molecules (determines specificity)



Hexokinase

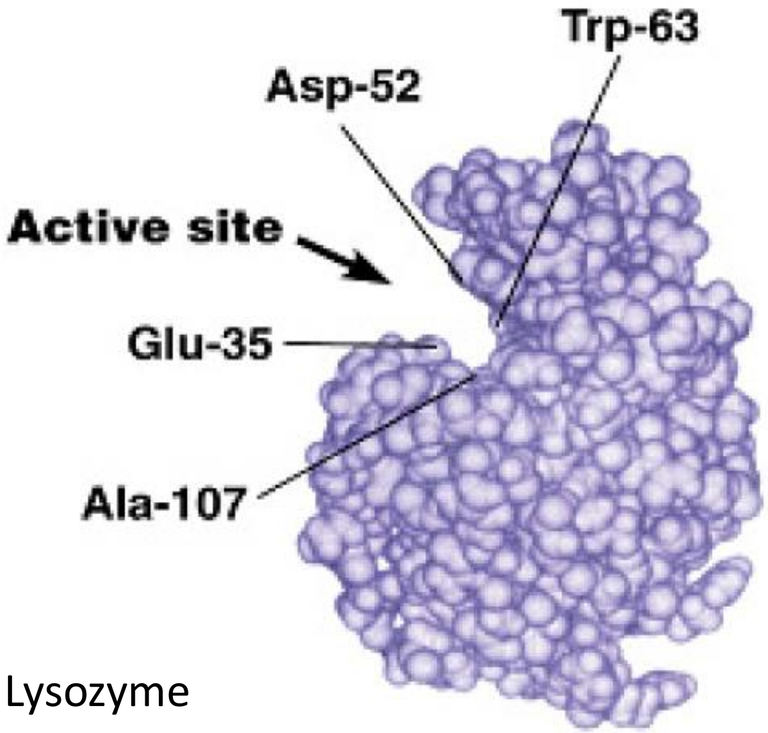


The first substrate here (glucose) is bound through induced fit and the second one (ATP) through lock and key

Features of active site 2

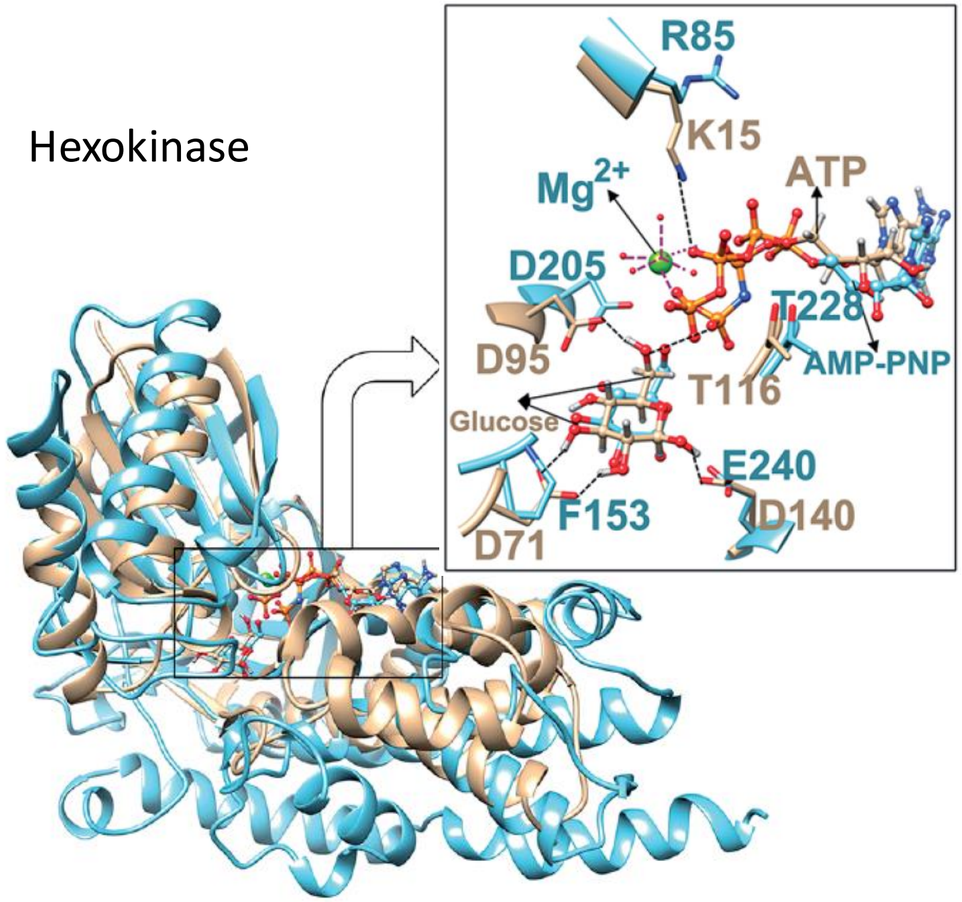
- It is a three-dimensional pocket formed by amino acid groups that come from **different parts of the primary structure usually forming a domain.**

Active site amino acids can come from far parts relative to the primary structure due to protein folding



Lysozyme

Hexokinase



Features of active site 3

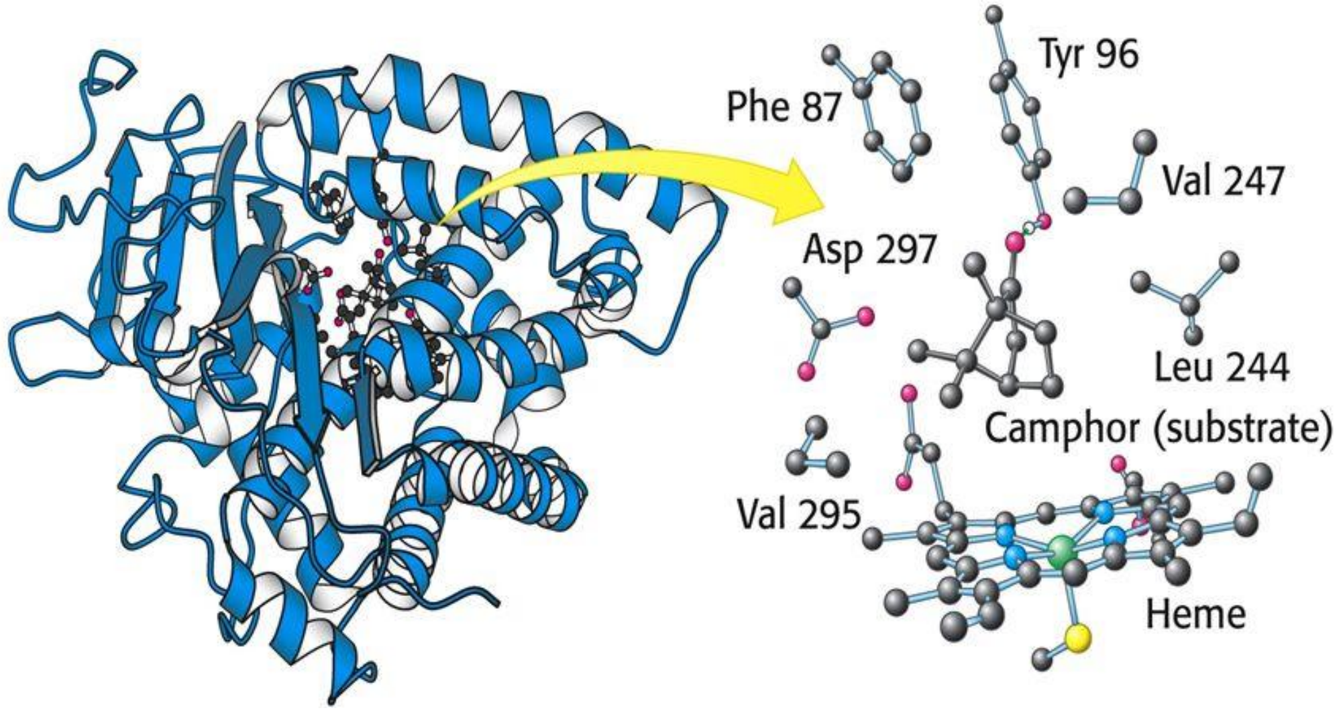
- The amino acid residues can be nonpolar and polar.
  - **Water is usually excluded unless it is part of the reaction.**
- Substrates bind to enzymes by multiple weak attractions initially.

Depending on the substrate

Because it breaks the non-covalent interactions (because of this, the active site is usually located internally)

Non-covalent

During the reaction, the substrate may bind to the active site via covalent bonds. At the end, these covalent bonds break down to release the product

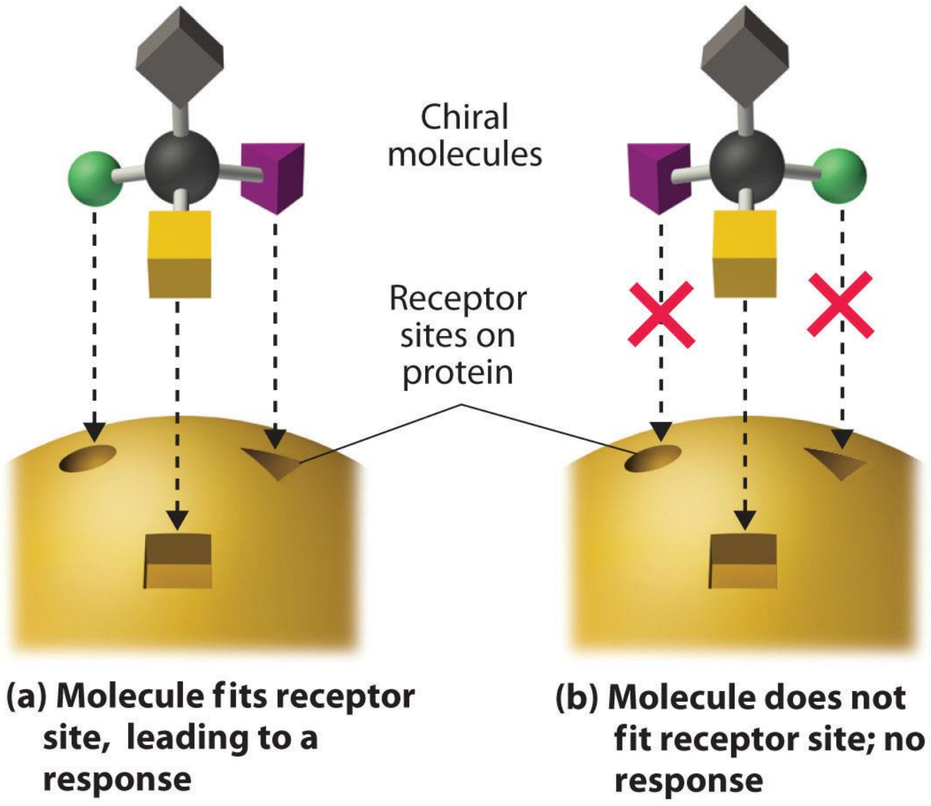


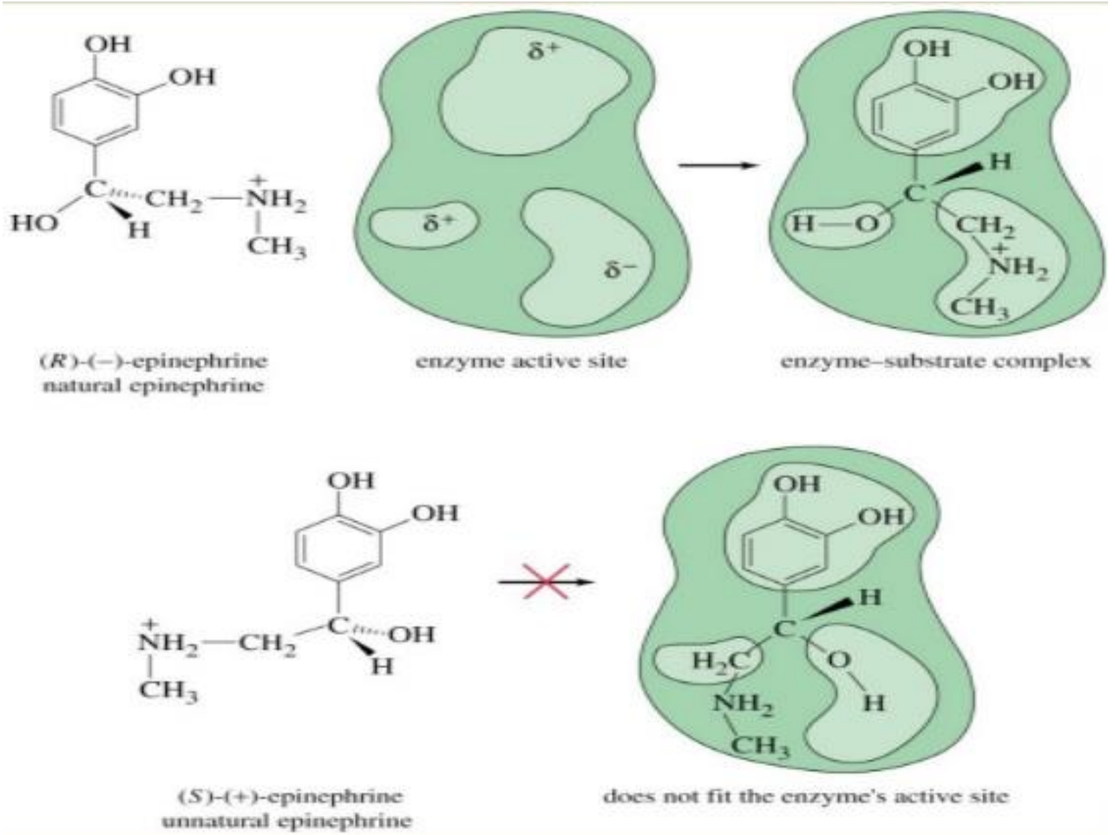
Features of active site 4

The interaction between the enzyme and the substrate at least 3 non-covalent interactions (3 points).

- Binding of substrates to active sites occurs at minimally three points.
  - **Chirality determines specificity.**

Example: Sucralose must be in a specific structure to give the sweet taste ,the enantiomer(mirror image) will give a sour taste.



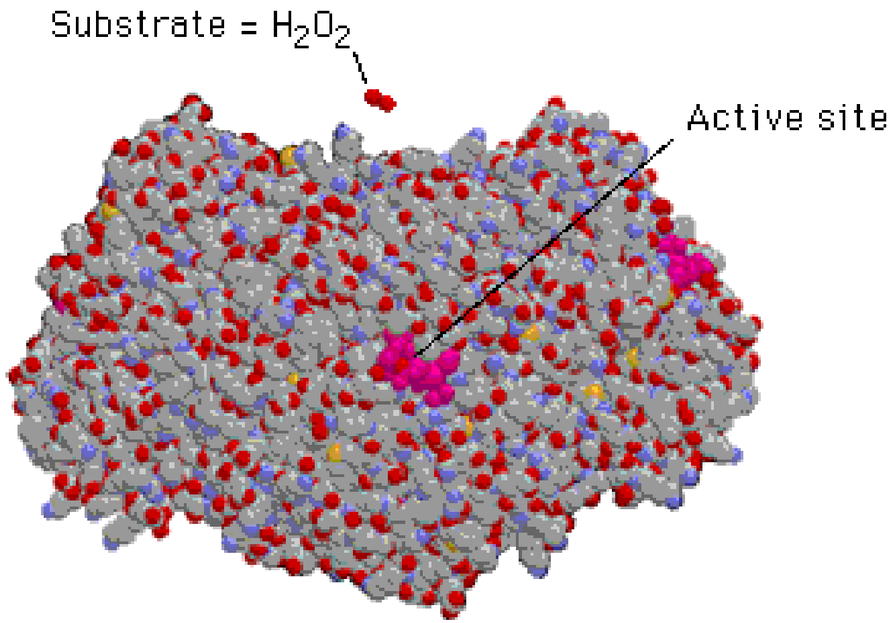


Features of active site 5

- It is small relative to the total structure of an enzyme.
- The “extra” amino acids **create the 3D shape of the active site.**

The remaining amino acids **may make up regulatory sites.**

Close/open the active site.



Molecular model of catalase

How do substrates fit into the active site of enzymes?

It is all random interactions (collisions) at the right angle so a reaction happens

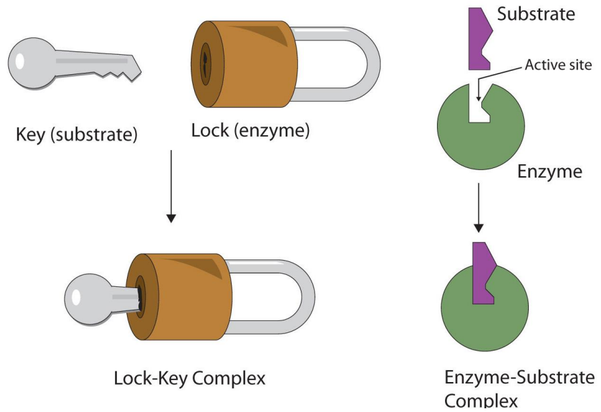
The two models

Some enzymes can be both. (slide 13)

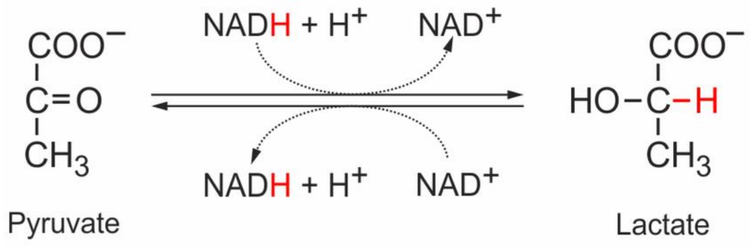
No changing in the shape of the active site.

Lock-and-key model

- Here, the substrate fits **directly** into the active site.



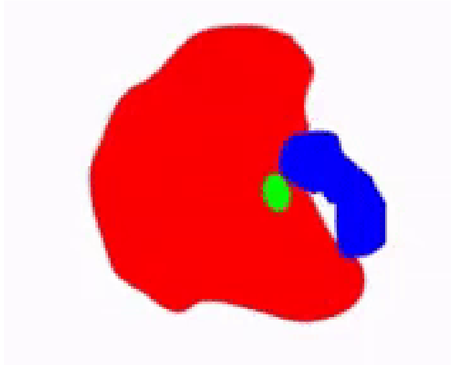
Example: Lactate dehydrogenase.



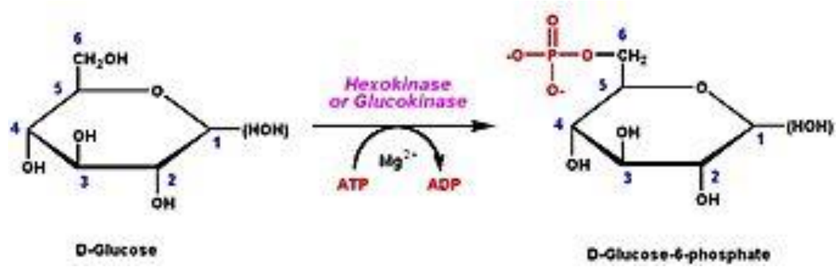
Induced fit model *(more favored)*

- Enzymes are flexible and active sites can be modified by binding of substrate.

Due to non-covalent interaction between the substrate and the active site (attraction/repulsion)



Example: Hexokinase



How do enzymes accelerate reactions?

Types of energy

- There are two forms of energy
  - potential - capacity to do work (stored) ---->Energy stored in bonds.
  - kinetic - energy of motion

- Potential energy is more important in the study of biological or chemical systems.
- Molecules have their own potential energy stored in the bonds connecting atoms in molecules.
  - It is known as **free energy** or **G** (for Josiah Gibbs).
  - It is the energy that is available for reactions to occur.

Free energy (G) and reaction types

- The difference between the free energy values between reactants and products (free-energy change ΔG):

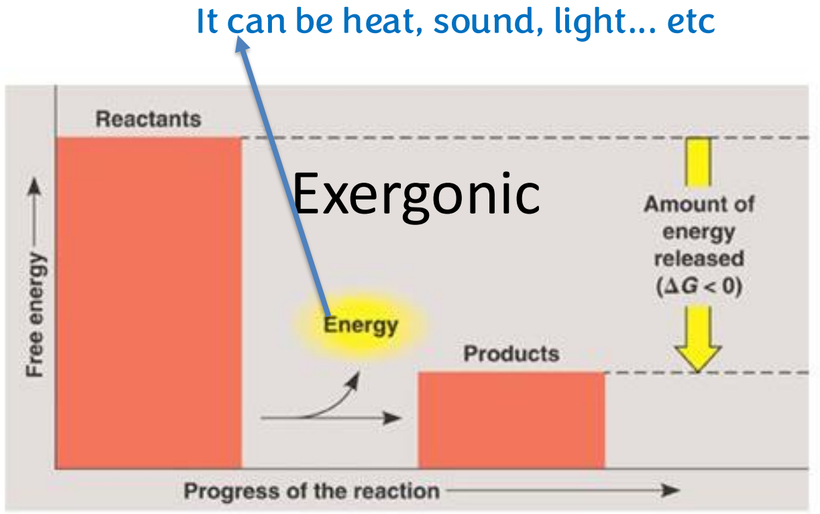
$$\Delta G = G_{products} - G_{reactants}$$

- Reactions are **exergonic, endergonic, or at equilibrium.**

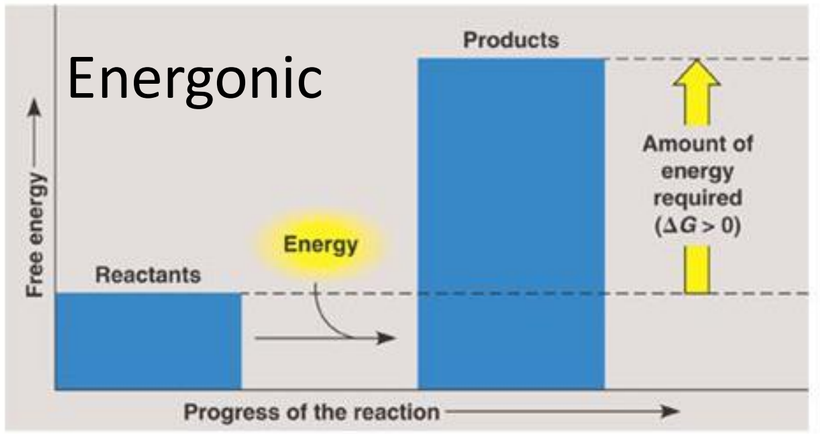
*Bond Energies, *D*, (kJ/mol)	
C—H	414
C—C	348
C—N	293
C—O	351

Do not memorize

Fats store more energy than sugars because they are highly reduced (rich in C–H bonds relative to sugars).



(a) Exergonic reaction: energy released



(b) Endergonic reaction: energy required

What does it mean?

$$\Delta G = G_{products} - G_{reactants}$$

- If ΔG is negative, $G_{products}$ is less than $G_{reactants}$, energy is not needed to drive the reaction, but is released, making the forward reaction (from left to right) spontaneous (the reaction is called exergonic).

Favorable

- If ΔG is positive, $G_{products}$ is more than $G_{reactants}$, an input of energy is needed, making the forward reaction not spontaneous (the reaction is called endergonic).
  - The reverse reaction is exergonic and , thus, spontaneous.
- If **ΔG is zero, both forward and reverse reactions occur at equal rates; the reaction is at equilibrium.**

	Exergonic	Endergonic
What happens to the energy?	Released	Absorbed
ΔG	Negative	Positive
Energy of reactants	Higher than products	Lower than products
Input of energy	Not required	Required
Spontaneity	Spontaneous/favorable	Unspontaneous/unfavorable
Exothermic or endothermic	Exothermic	Endothermic

Spontaneous vs. instantaneous

- **<u>Spontaneous means naturally occurring, and instantaneous means occurring right away.</u>**
  - The term spontaneous in chemistry has nothing to do with how fast or slow a reaction or process takes place. It concerns the direction in which a reaction or process *can* change, **given a set of starting conditions.**
- The simplest examples are phase changes, such as water going between liquid and solid. For water, we can write:

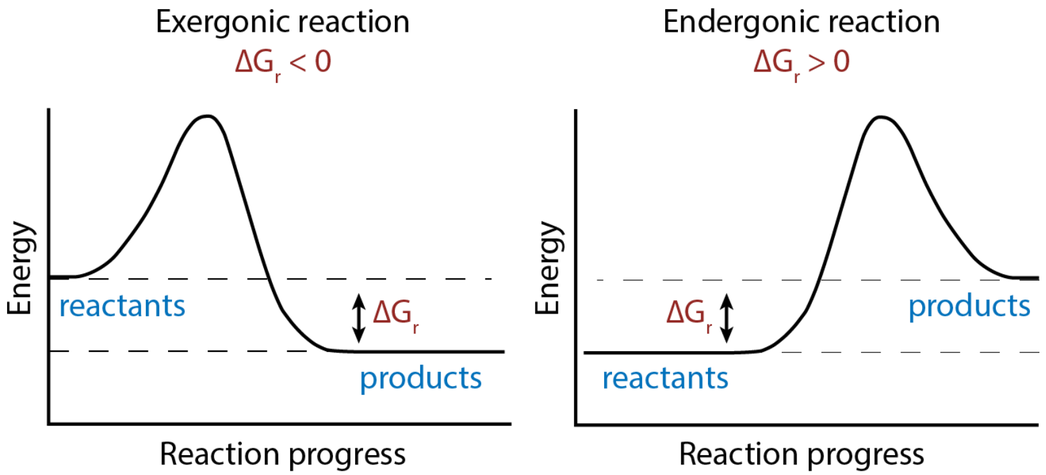
$$H_2O_{liquid} \rightleftarrows H_2O_{solid}$$

- Below 0°C, the equilibrium lies to the right, so we would say the left-to-right process is spontaneous, whereas the right-to-left process is non-spontaneous.
- Above 0°C, the right-to-left process is spontaneous, whereas the left-to-right process is non-spontaneous.
- At the phase transition temperature, 0°C, left and right are equally favored, so the reaction is at equilibrium.

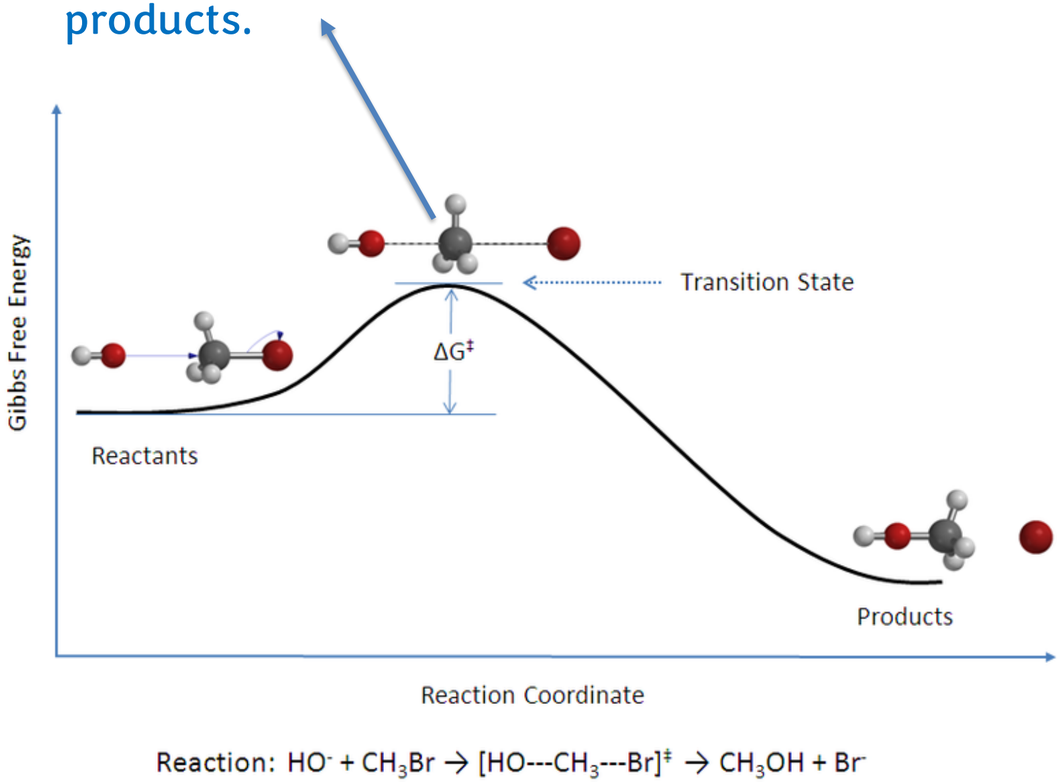
Transition state

Enzyme–substrate complex

- **Any reaction** goes through a transition state molecule (ES) that has a higher free energy than does either S or P.
- The difference in free energy of the transition state and the substrate is called the activation energy.
  - The configuration of the transition state molecule is most unstable and has the highest activation energy.



The transition state requires more energy because it's unstable, turning quickly into products.

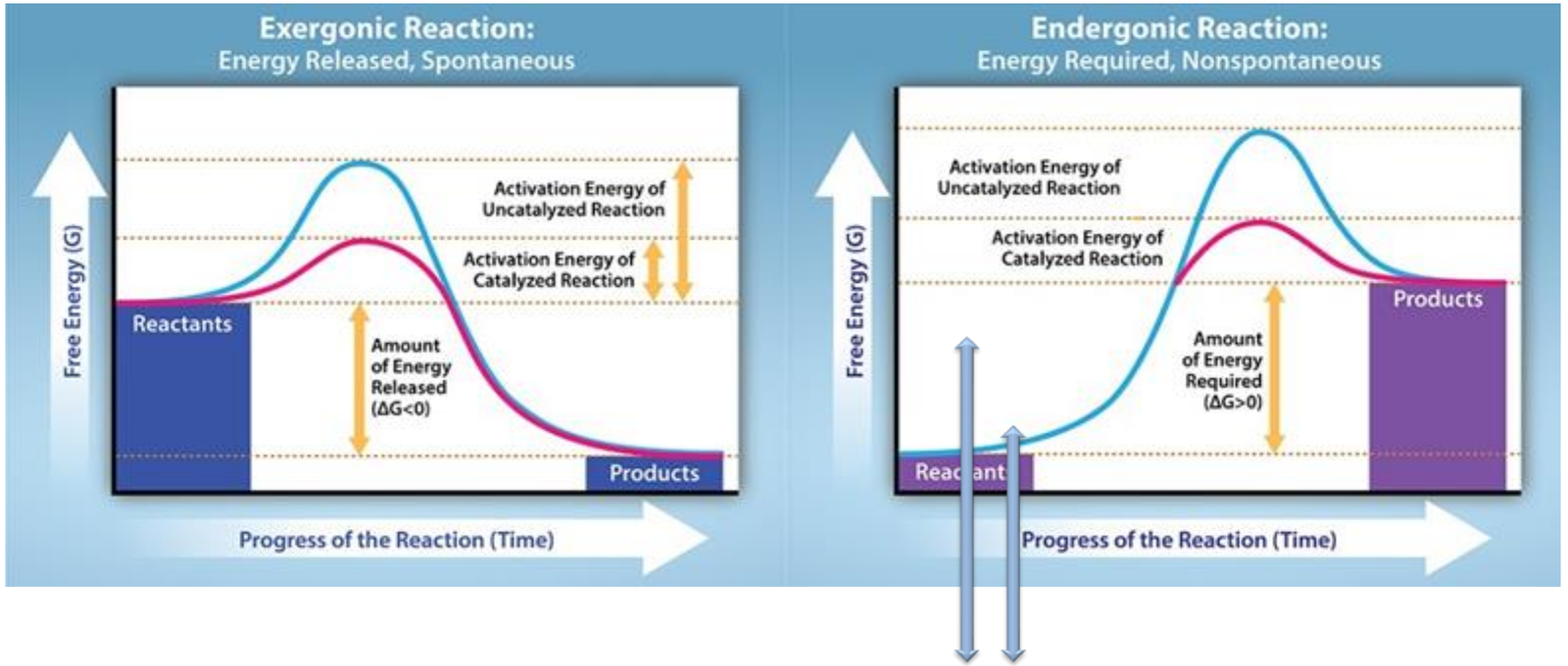


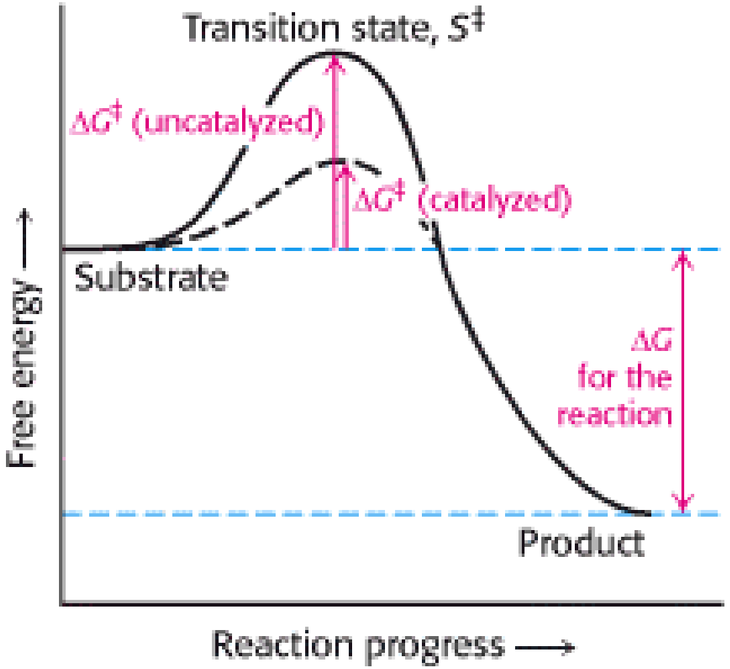
Reaction: $HO^- + CH_3Br \rightarrow [HO{---}CH_3{---}Br]^{\ddagger} \rightarrow CH_3OH + Br^-$

The free energy of the transition state is higher than that of the substrate or the product.

What do enzymes do?

- Enzymes **lower the activation energy of the transition state** or, in other words, enzymes facilitate the formation of a transition state at a lower energy.

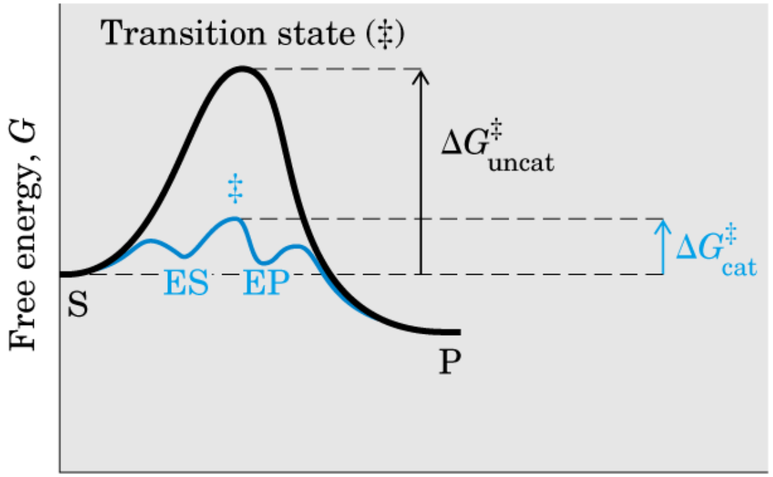




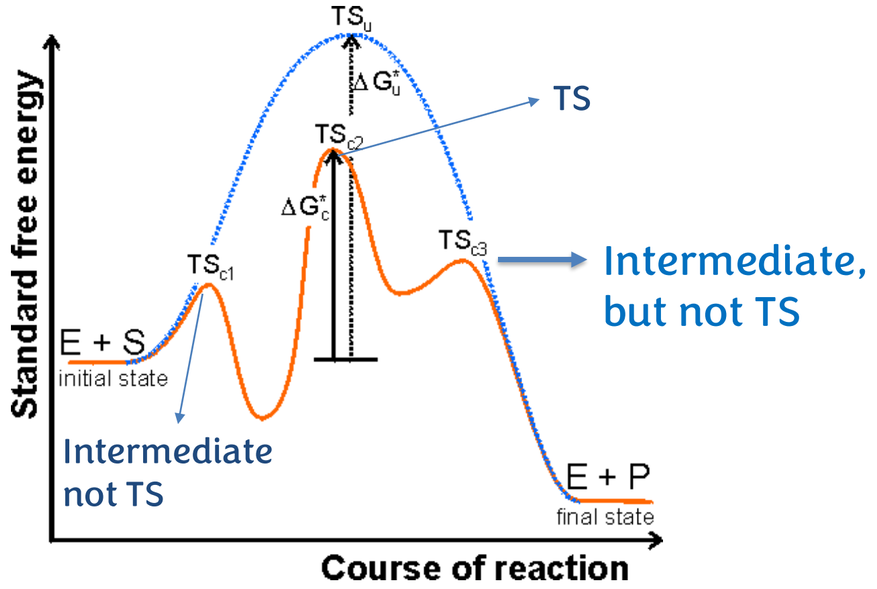
ΔG,G-product/Substrate, do not change .

Alternative pathways

- Substrates often undergo **several transformations when associated with the enzyme** and each form has its own free energy value.
- The activation energy corresponds to the intermediate with the highest free energy.
- The energy of activation does not enter into the final ΔG calculation for a reaction.
  - ΔG of the reaction does not change.

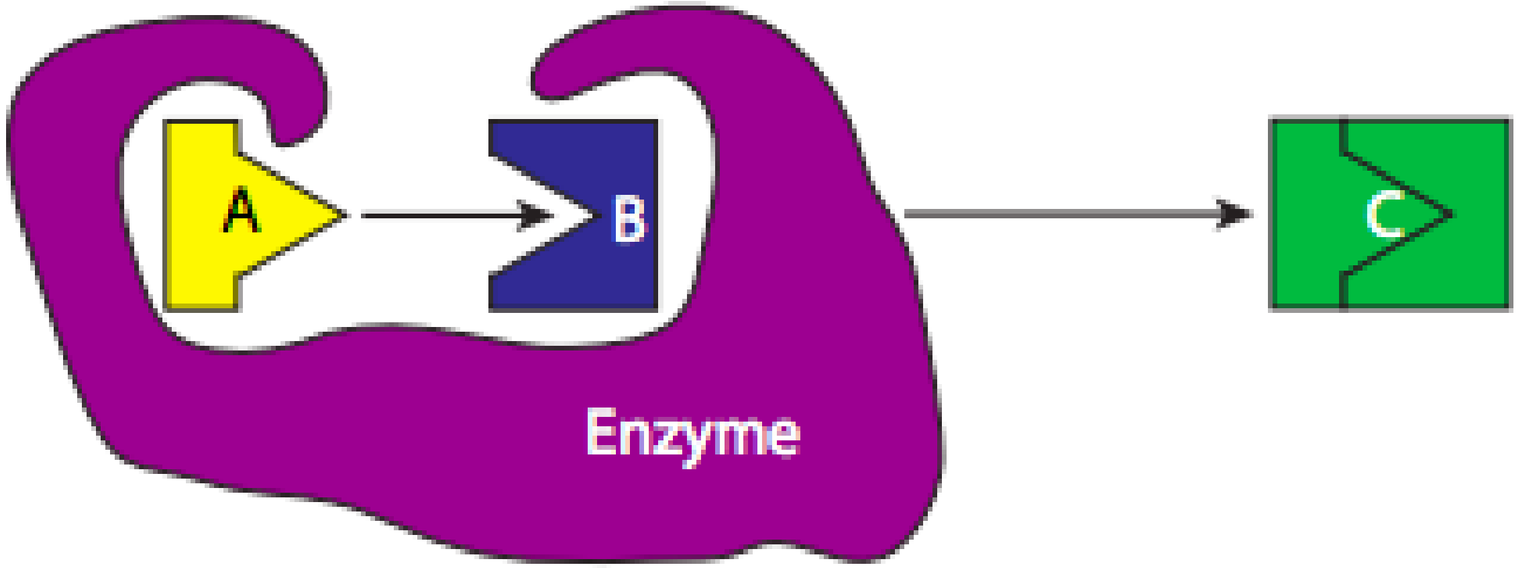


The intermediate with the highest energy is the transition state.
Activation energy: TS energy- S energy .



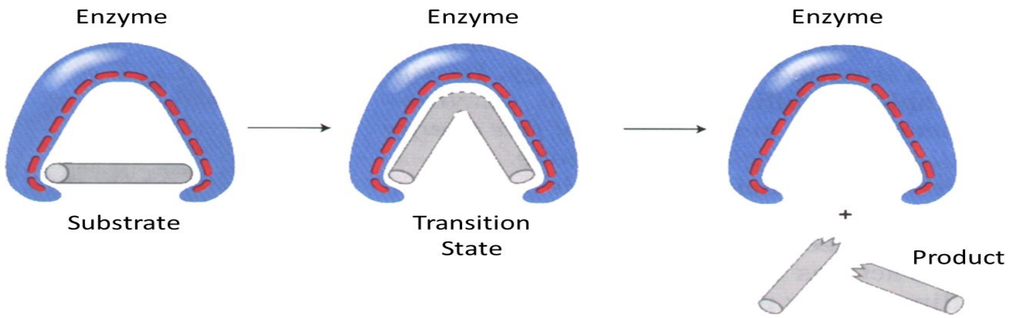
How do enzymes catalyze reactions?

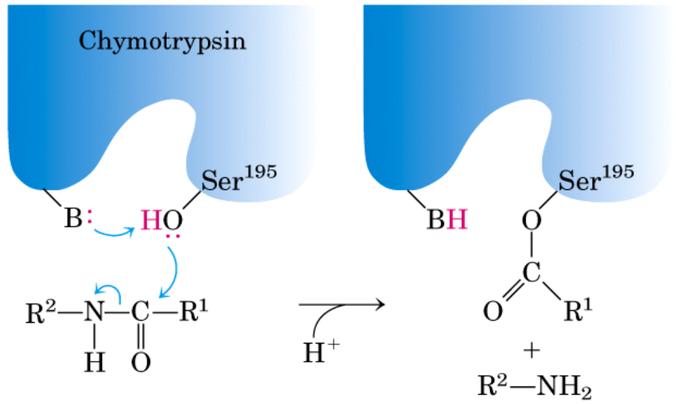
- Enzymes place substrates **proximal** to each other.
- Enzymes **orient** the substrates inside the active site in the best possible fit.



Catalytic strategies

- Enzymes use different strategies to catalyze reactions such as:
  - **Straining the bonds** within the **substrates**, making them **easier to break**
  - Driving **acid/base reactions**, usually involving a proton donor/acceptor such as histidine, serine, etc.
  - **Covalent catalysis**, where the substrate is covalently linked to an amino acid side chain at the active site of the enzyme during the course of the reaction.
  - Metal-Ion Catalysis, where **enzymes contain metal ions** to drive catalysis.
  - Cofactor Catalysis, where a **cofactor is needed for an enzyme to form a covalent bond with the substrate** during the course of the reaction.





Quiz!

For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1			
V1 → V2			

Additional Resources:

رسالة من الفريق العلمي:

Reference Used:

Prof. Mamoun Ahram recorded lecture

Extra References for the Reader to Use:

Marks’ Basic Medical Biochemistry (6th edition)