

Biomedical Science

Molecular Genetics



BMS 23110c | Fall 2026-2027

Enzymology

The Mechanism Of Enzyme Action II

جميع الأوراق والمحتويات التابعة للمنصة محمية بموجب حقوق الملكية الفكرية.
يُمنع منعاً باتاً نسخ، أو نشر، أو مشاركة أي محتوى أو مستندات تخص المنصة بأي شكل
دون إذن مسبق. ويُعد أي تجاوز أو مخالفة لهذا القرار خرقاً قانونياً يعرض مرتكبه للمساءلة
والملاحقة القانونية الفورية

A+ is more than just a platform



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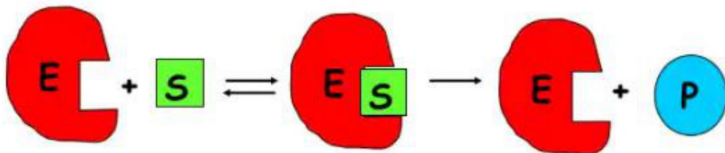


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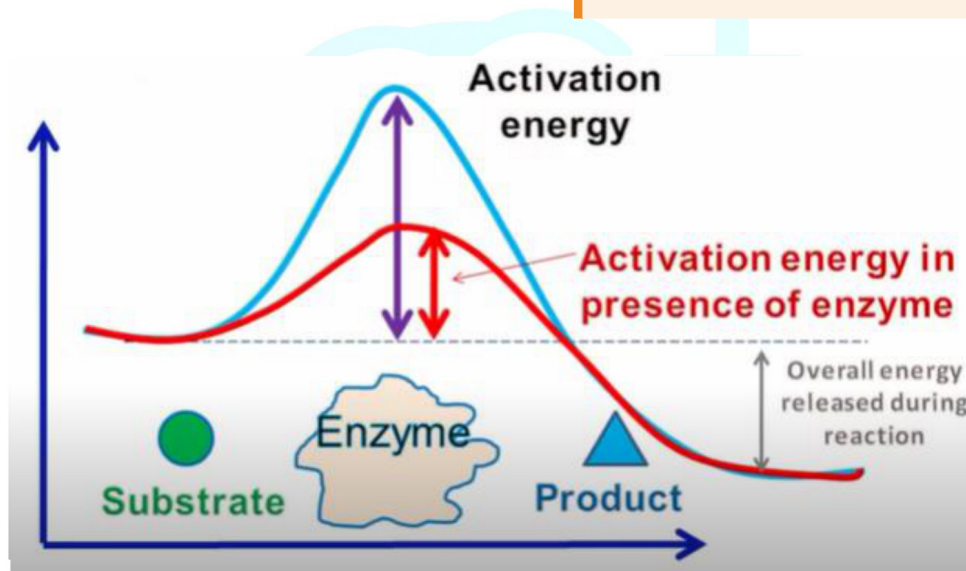


How Do Enzymes Work?

An **enzyme (E)** is typically a protein molecule that promotes a reaction of another molecule, its **substrate (S)**. This binds to the active site of the enzyme to produce a transition **enzyme-substrate complex ES**, and to form a **product P**.



The overall reaction is composed of **two elementary reactions** in which the substrate forms a complex with the enzyme that subsequently decomposes to products and enzyme.



TO DEDUCE THE MECHANISM OF AN ENZYME-CATALYZED REACTION, WE SHOULD DETERMINE

01

Sequence

The sequence of the enzyme-containing complexes as substrate(s) is (are) converted to product(s)

02

Rates

The rates at which these complexes are interconverted

03

Structures

The structures of these complexes

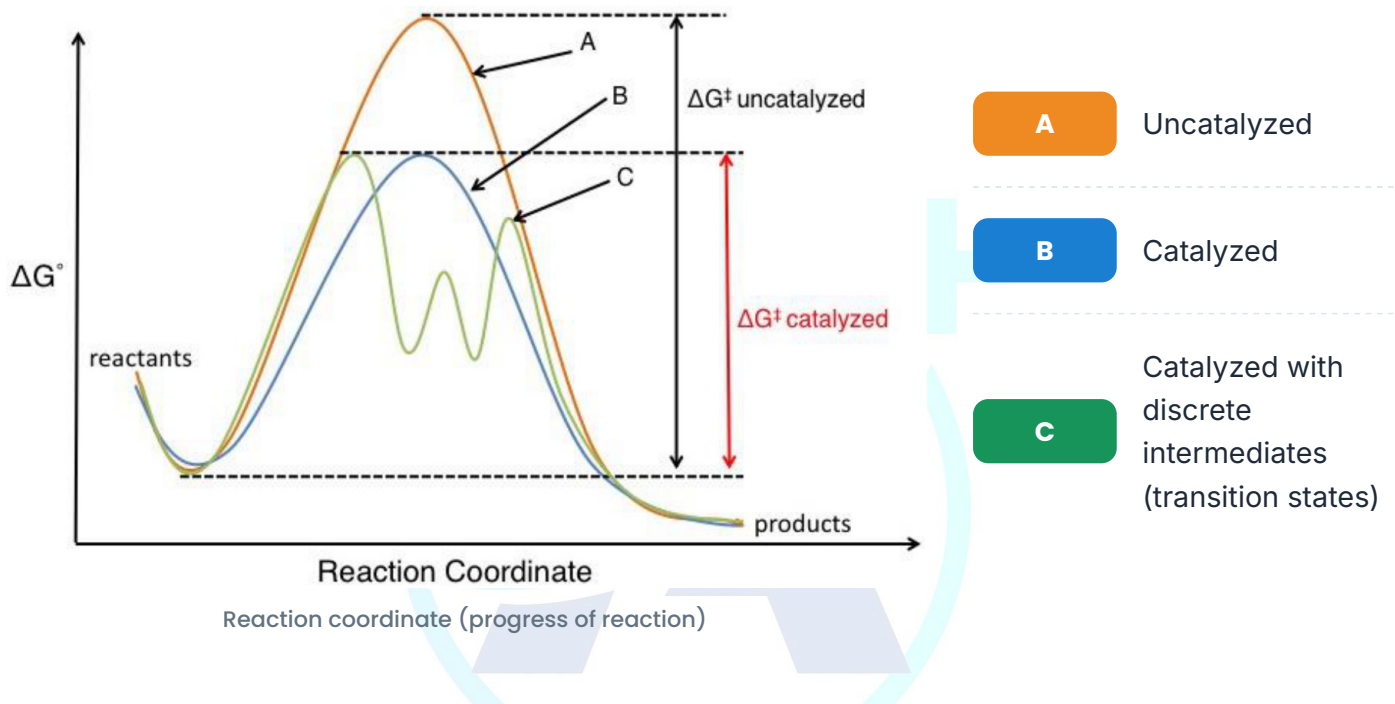
Kinetic and **structural** techniques have made major contributions to our understanding of enzyme mechanisms.



The Energy Profile

Various factors contribute to the **enhancement of the rate** of reactions.

The **"Energy profile"** for a typical reaction shows that in order to proceed from reactant(s) to product(s) an **energy barrier (ΔG)** must be surmounted.



In order to speed up the rate of a reaction at **constant temperature**, the value of ΔG **must be reduced.**

ENZYMES



Transition state

Intermediate formed between the initial chemical reactant and the product.



Must pass

For substrate (reactant) to become product (catalyzed or not), the substrate **must pass** through the transition state.



Lower barrier

Enzymes **reduce the free energy of activation** of the reactions they catalyze.

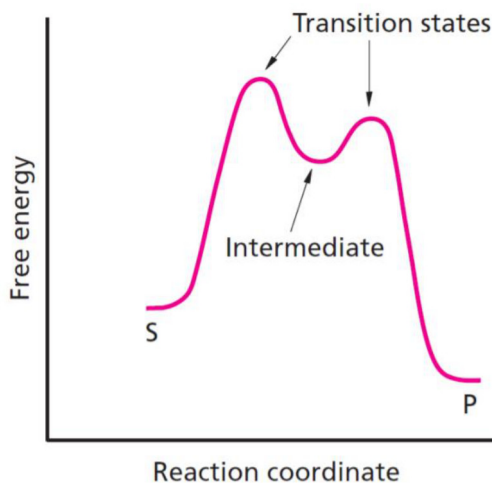


Stabilization of the Transition State

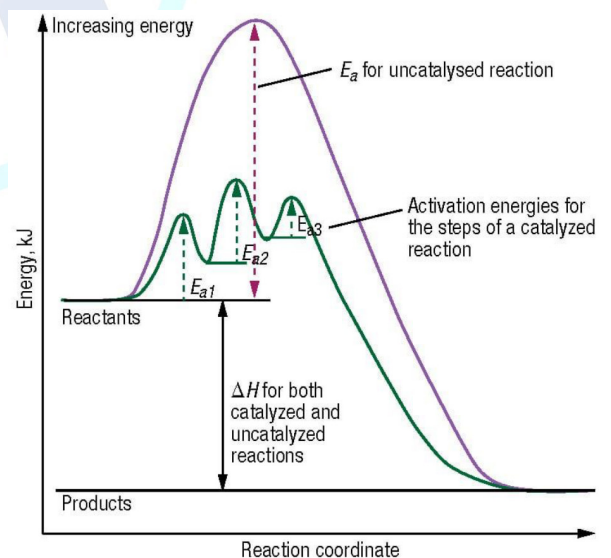
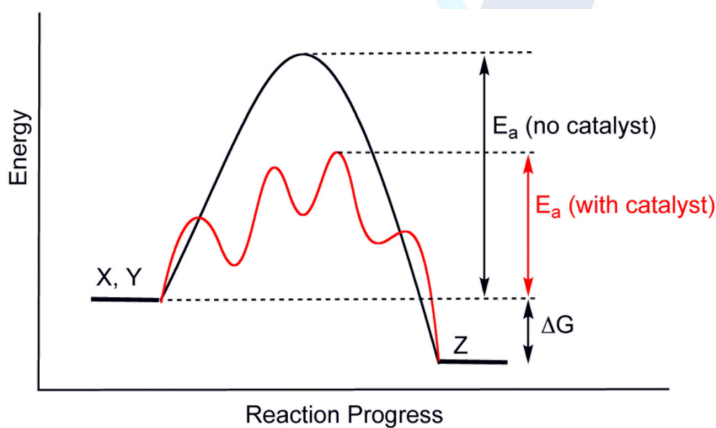
KEY IDEA

Stabilization of transition state is the mechanism of enzyme action.

Transition State Vs. Intermediate



The **highest point** of the energy profile is designated the **transition state** of the reaction.

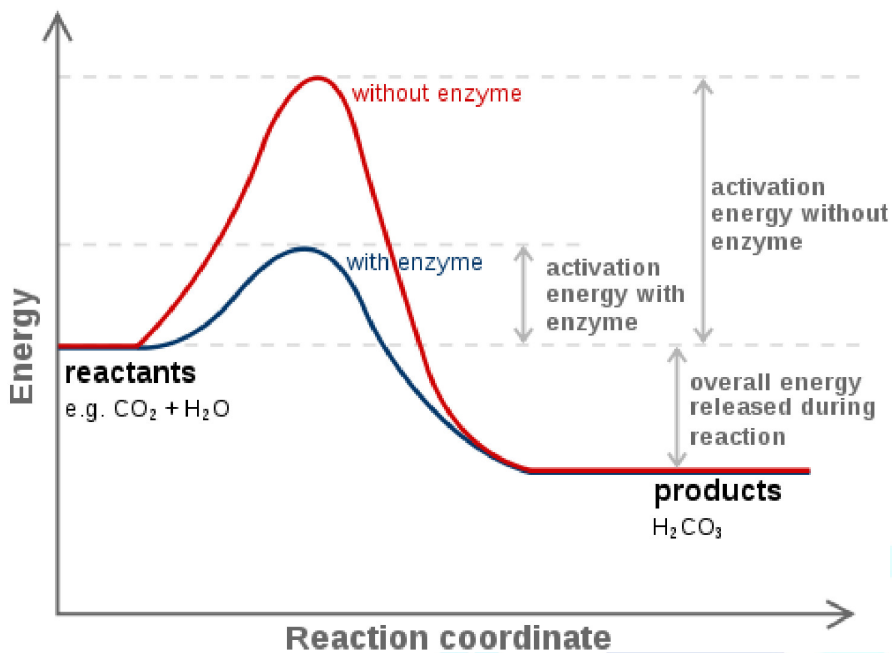


FUNCTION OF A CATALYST

The function of a catalyst is to provide an **alternative reaction pathway** so that a lowered energy barrier has to be surmounted.



Example: Carbonic Anhydrase



Carbonic anhydrase, which is found within **red blood cells**, catalyzes a reaction converting **CO₂ and water** into carbonic acid.

LECTURE VIDEOS

youtube.com/watch?v=ie_7Y7HJlps
youtube.com/watch?v=m_9bpZep1QM



Factors Enhancing the Rate of Reactions

Various factors contribute to the enhancement of the rate of reactions:



Proximity & orientation effects

Substrates brought close together and guided into the active site in a specific orientation.



Acid–base catalysis

Proton (H⁺) donors and acceptors stabilize developing charges.



Covalent catalysis

Substrates form a transient covalent bond with active-site residues.



Changes in environment

Reaction rates are highly sensitive to the nature of the solvent.



Proximity & Orientation Effects



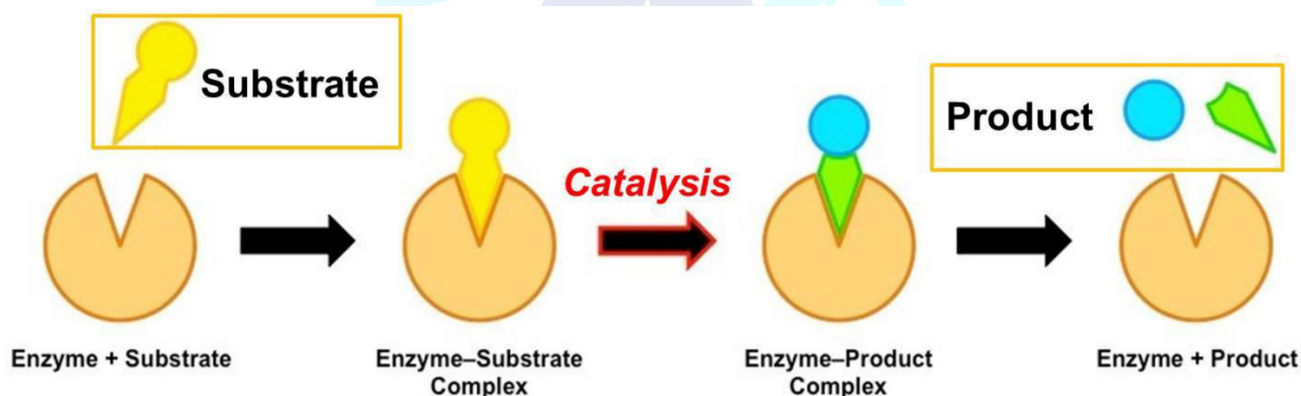
Proximity

- The **higher the concentration** of enzymes and substrates, the **more frequently** they will encounter one another.
- An enzyme could increase the rate of a reaction involving more than one substrate by **binding the substrates at adjacent sites** and therefore bringing them into close proximity with each other.

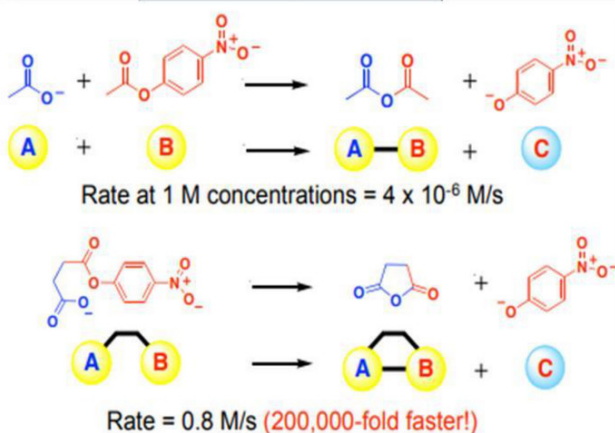


Orientation

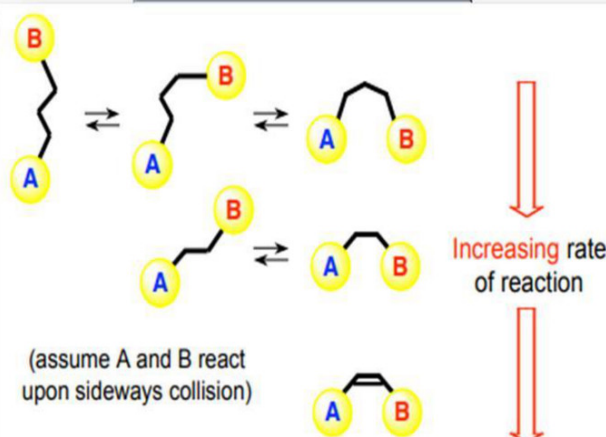
A close proximity between a substrate and an enzyme active site does **not** mean that a catalysis reaction will occur. The enzyme must **guide or steer the substrate** into the active site in a specific orientation to make the reaction actually occur, which is called **orientation**.



Proximity effects



Orientation effects



Proximity effects and orientation effects on reaction rates.



Acid–Base & Covalent Catalysis

⇌ Acid–base catalysis

- Anything that can **stabilize the charges on the intermediate** and hence the developing charges in the transition states will **lower the energy of the transition state** and catalyze the reaction.
- Many reactions of the type catalyzed by enzymes are known to be catalyzed by **acids and/or bases**.
- The **acid** is the proton (hydrogen ion, H^+) **donor** and the **base** is the proton **acceptor**. Typical reactions catalyzed by proton transfer are **esterifications** and **aldol reactions**.

🔗 Covalent catalysis (intermediate formation)

Catalysis which involves substrates forming a **transient covalent bond** with the residues present in the active site is referred to as **covalent catalysis**.

If the catalysis involves **participation of small organic molecules, co-factors and amino-acid side chains from the enzyme**, it is referred to as the **lock and key model**.

If the catalysis involves **water molecules** for proton donation or acceptance, the catalysis is referred to as **acid–base catalysis**.

💧 Changes in environment

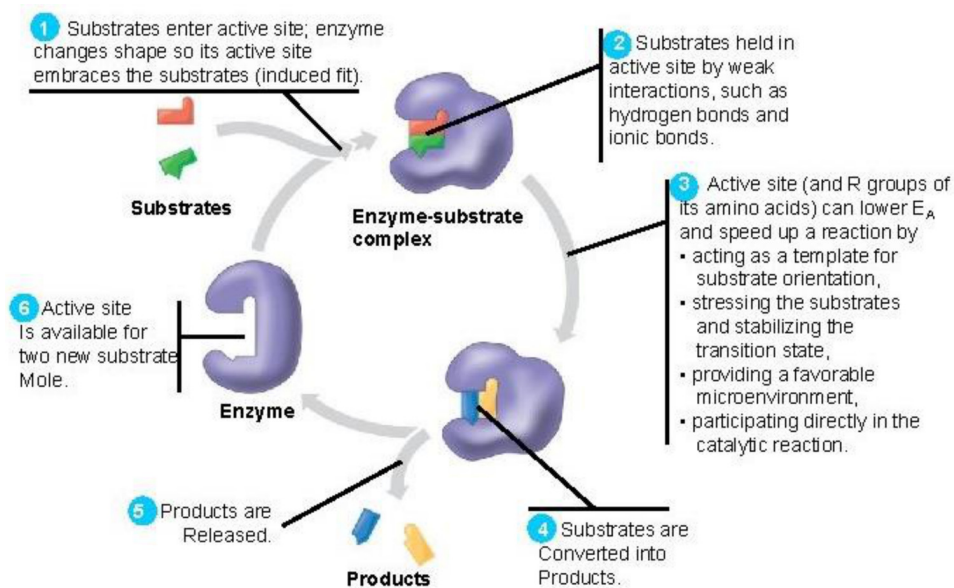
The rates of many organic reactions are **highly sensitive** to the nature of the **solvents** in which they occur.

⚠️ Strain or distortion

The bonds in a substrate may be **distorted** on binding to the appropriate enzyme. This would speed up the reaction if the distortion (or strain) **lowered the free energy of activation** by making the geometry and electronic structure of the substrate more closely resemble that of the postulated transition state.



The Catalytic Cycle of an Enzyme



Check Yourself

Q1

The target substrate molecules bind to active site of the enzyme transforming into products through a series of steps known as the ____

☐ a enzyme kinetics

☒ b enzymatic mechanism

☐ c chemical kinetics

☐ d zero order reaction kinetics

Q2

If the catalysis involves participation of small organic molecules, cofactors, and amino-acid side chains from the enzyme; it is termed as ____

☐ a specific acid base catalysis

☐ b general acid base catalysis

☐ c substrate collision theory

☒ d lock and key model

Q3

____ involves substrates forming transient covalent bond with the residues present in the active site

☒ a Covalent catalysis

☐ b Specific acid-base catalysis

☐ c General acid-base catalysis

☐ d Substrate catalysis



Experimental Approaches

Experimental approaches that can be used to **elucidate the mechanism** of action of an enzyme:

- 1 Kinetic studies
- 2 Detection of intermediates
- 3 X-ray crystallography
- 4 Chemical modification of amino-acid side chains
- 5 Site-directed mutagenesis

These approaches are to be regarded as **complementary**.

The information gained from one approach should be interpreted in the light of results from the others in order to build up the **overall picture** of the mechanism.



Kinetic Studies



What they cover

1. How the catalytic activity of an enzyme could be analyzed
2. Principal concepts and equations of enzyme kinetics



Why they matter

How **information about enzyme mechanisms** can be gained from these studies.



Information from Kinetic Studies

THE TYPES OF INFORMATION ON ENZYME MECHANISM AVAILABLE FROM KINETIC STUDIES

EXPERIMENT	INFORMATION AVAILABLE
Variation of the substrate concentration(s)	Sequence of complexes in reactions Distinction between possible mechanisms
Variation of the substrate structure	Structural features responsible for binding and catalytic activity (mapping active site)
Reversible inhibition	Competitive inhibitors can help to define the active site
Variation of pH	K_a values of the side-chains involved in the catalytic activity. Assignment of the K_a to particular amino-acid side-chains may be possible
Pre-steady-state kinetics	Detection of the enzyme-containing complexes Rates of the elementary steps in the reaction

CONSTANTS & TIMESCALES



K_a — association constant

Measures the ratio of enzyme–ligand to free enzymes and free ligands. This measures **only binding affinity**.



K_d — dissociation constant

Measures the **inverse**. This also only measures binding affinity.



K_m

Takes into account that enzyme–ligand complexes can **react to form products**, and so adjusts for enzyme binding and reacting. The amount of substrate needed for enzymes to be reacting at half max velocity.



Pre-steady-state kinetics

In the first moment after an enzyme is mixed with substrate, no product has been formed and no intermediates exist. The study of the **next few milliseconds** of the reaction is called pre-steady-state kinetics.



Detection of Intermediates

One of the most **direct methods** for obtaining information about the pathway of a reaction is to

detect any intermediates that may be involved in the reaction.

It may be possible to **stabilize intermediates at low temperatures**, where the rate of breakdown will be decreased.



Transition State vs Intermediate

WHAT IS THE DIFFERENCE?

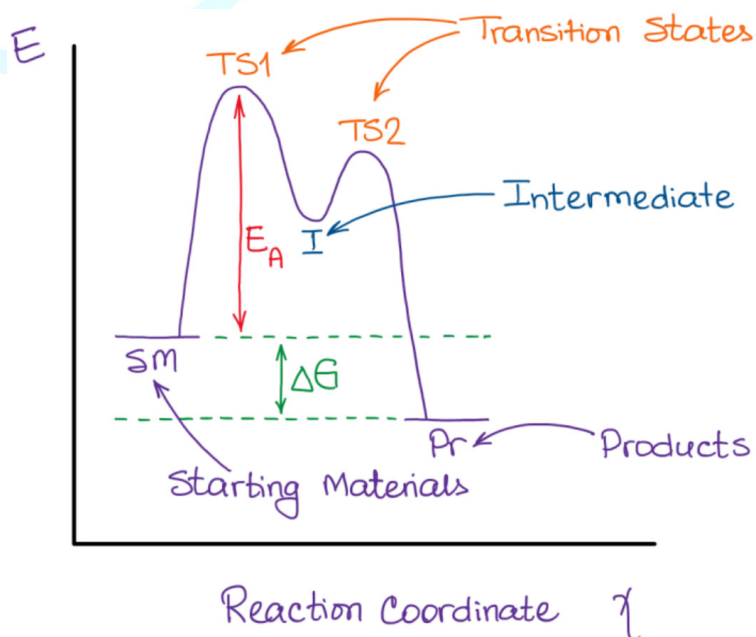
Understanding the difference between the transition state and an intermediate will help you in **drawing the mechanisms**, explaining the mechanistic differences, and understanding what exactly is going on in the reaction.

READING THE DIAGRAM

x The **x-axis** is a reaction coordinate: a loosely defined term meaning the reaction progress in the general direction from the starting materials or reagents (**SM**) to the products (**Pr**).

E The **energy curve** describes the energy states of the components at a certain point in the reaction.

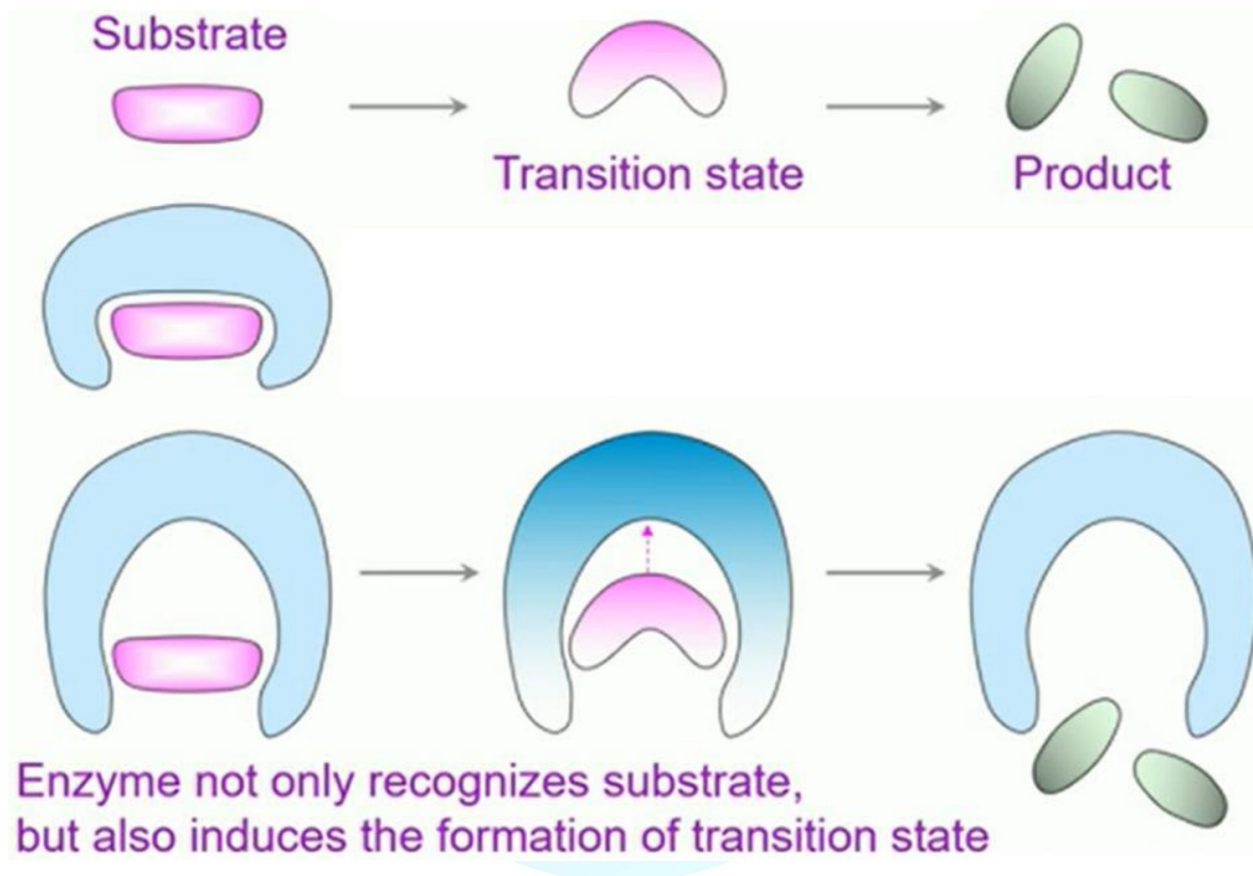
‡ The **transition states (TS)** and an **intermediate (I)**.





Definition of a Transition State

Transition state is the **highest point** (or points) on the reaction coordinate diagram. Those are the **"peaks"** or the **"hills"** in the picture.



THE TRANSITION STATE

Neither

It's neither the reactant nor is it a product

Resembles

It resembles both to some extent

Not isolable

It's not something that can be isolated (exists only a vibration-long)

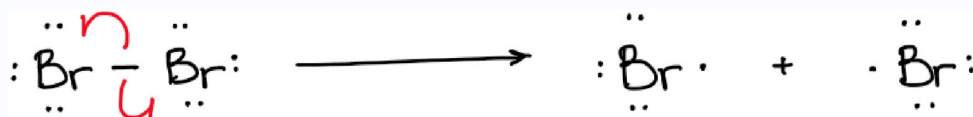
A transition state for **every single step** in your reaction. So, you can always check the number of transition states by counting the steps in the mechanism and vice-versa.

A more strict definition: a transition state is a molecular entity that has a **lifetime no longer than a vibration** that exhibits some structural characteristics of **both the reactants and the products**.

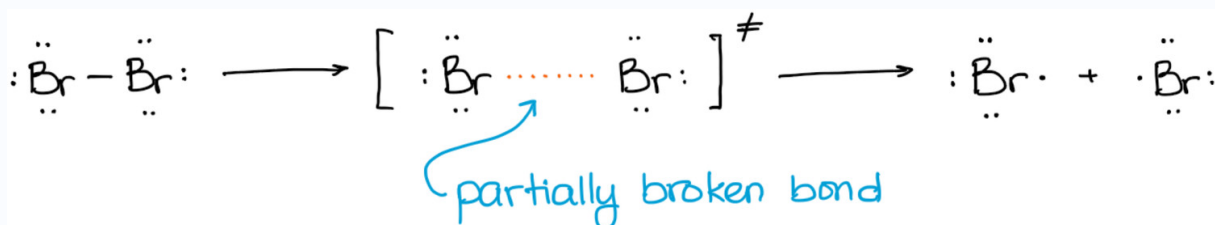


Every Reaction Has a Transition State

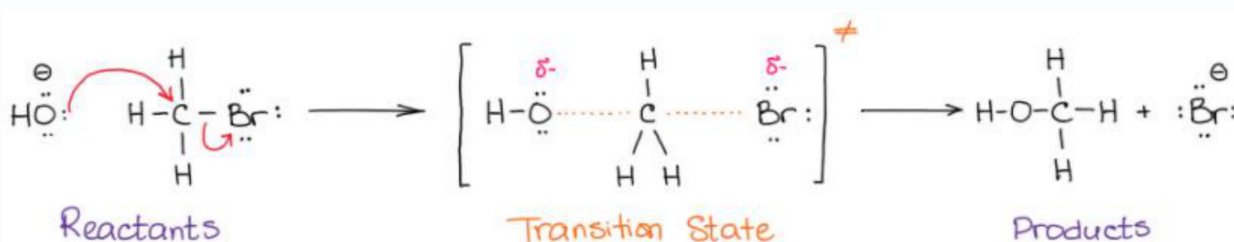
Every reaction, no matter how simple it might be, has a transition state. For instance, consider the following **bromine dissociation** reaction giving two bromine radicals:



The two bromine atoms didn't just magically split and appear in different places in space. They first had to **stretch the bond** to the brink of breaking and then break apart:

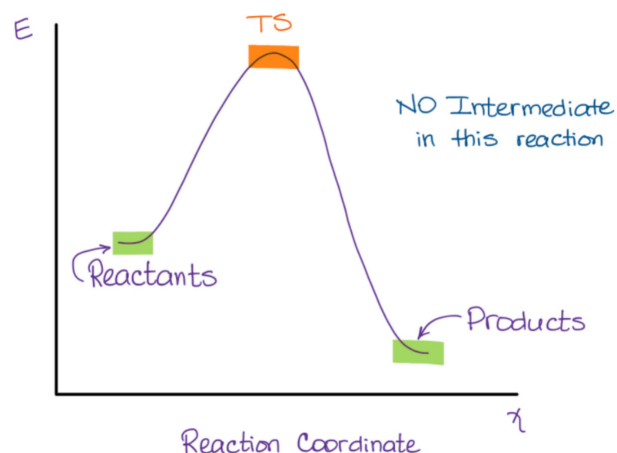


A REACTION WITH NO INTERMEDIATE



No intermediate is formed. You do have a **transition state** though, in which the O-C bond is not quite formed yet and the C-Br bond is not quite broken.

The energy diagram for this reaction →





Definition of an Intermediate

An intermediate is a comparatively **long-lived species** that can be experimentally **detected and characterized**. This means that an intermediate is an actual molecule or an ion that you can work with, sometimes even isolate.

Long-lived

A comparatively long-lived species

Detectable

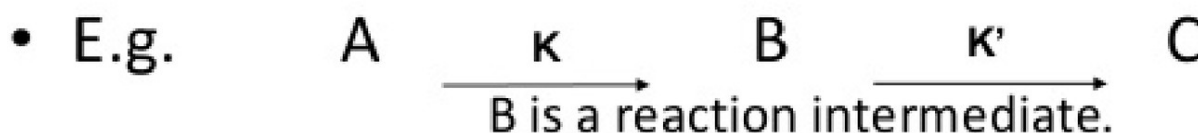
Can be experimentally detected and characterized

Stable

It is a relatively stable midpoint (intermediate)

Complex Reaction:

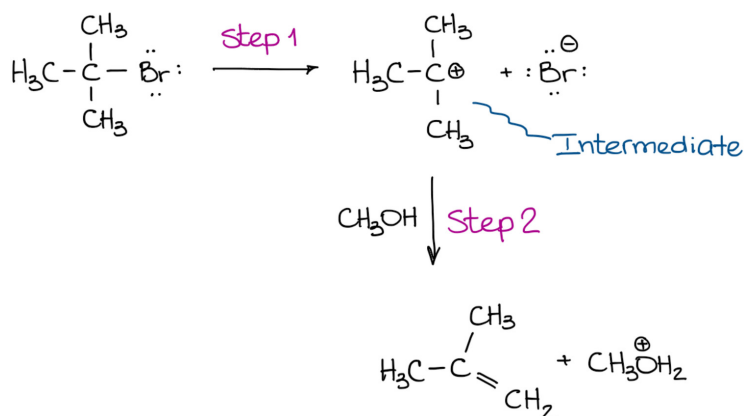
- It is a Multi-step reaction.
- These are reactions involving simultaneous breakdown by a sequence of reaction steps.
- Slowest step of complex reaction is called rate determining step.



It is also very important to remember that a reaction **doesn't have to have an intermediate**. You may or may not have one depending on the nature and the mechanism of the reaction.

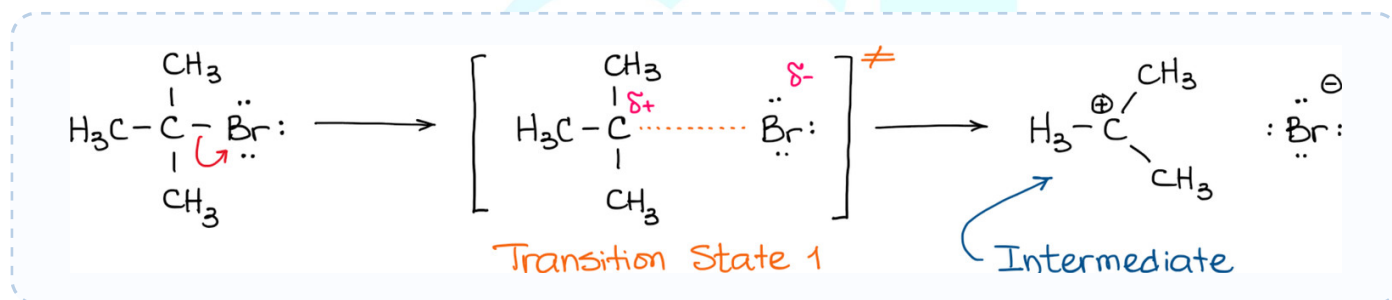


Two Transition States & One Intermediate



- In this reaction, an **intermediate** is formed.
- That intermediate is produced **before** the reaction proceeds into the next step.
- It is the intermediate that **reacts further** giving the final product.

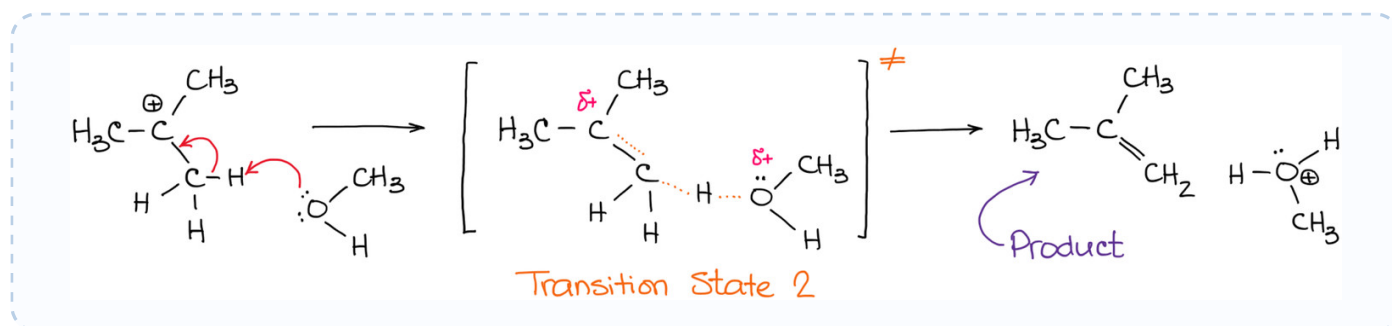
STEP 1 · TRANSITION STATE 1



The **first transition state** is the process of the C-Br bond elongation that leads to the eventual bond dissociation and the formation of the **carbocationic intermediate**.

The **intermediate** does exist long enough (although still very briefly) so that it is possible to "catch" it using experimental techniques and characterize it.

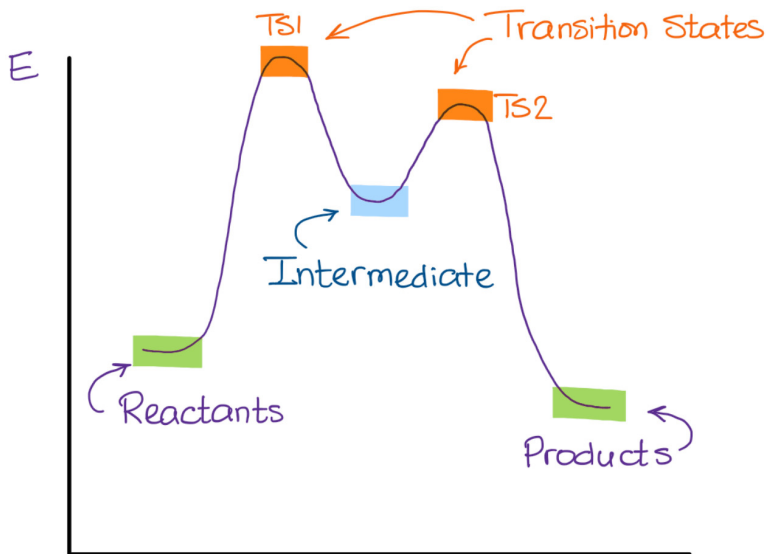
STEP 2 · TRANSITION STATE 2



The second step has its own transition state (2): there are three different bonds in the "making-breaking" state — the **H-O** and **C=C** bonds are forming, while the **C-H** bond is breaking.



Hilltops & Valleys



QUICK RULE-OF-THUMB

To identify the transition states and intermediates in a reaction, look for the **hilltops and valleys** on the diagram.

Reaction Coordinate

Plotting this reaction on the reaction coordinate vs energy plot

Hilltops

Transition states

Valleys

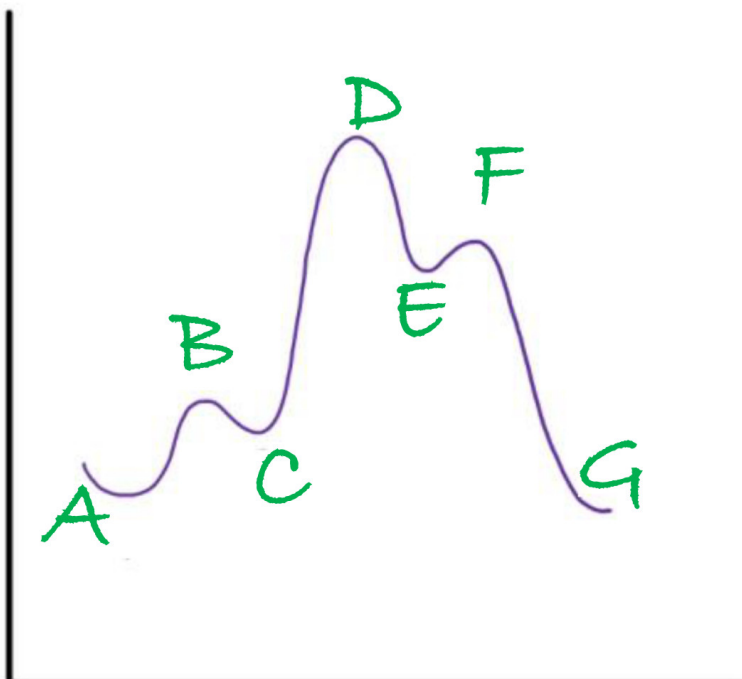
Intermediates

Beginning

Reactants

End

Products



The **hilltops** are the transition states and the **valleys** are the intermediates. The beginning of the curve is the reactants and the end is your products.



X-ray Crystallographic Studies

X-ray crystallography gives a **wealth of detail** about the structures of enzymes.

Up to fairly recently it has not been generally possible to study the structure of the catalytically active complex of enzyme and substrate(s) directly, because the collection of diffraction data takes **several hours**, by which time conversion of substrate(s) to product(s) will have occurred.

IT MAY BE POSSIBLE TO EXAMINE THE ACTIVE COMPLEX

- 1 **First**, in the case of a one substrate reaction when the equilibrium lies very much to one side.
- 2 **Second**, an enzyme can be studied in the presence of a **very poor substrate** or a **competitive inhibitor**. These molecules are likely to bind to the active site in a manner similar to the substrate but will remain essentially unchanged over the time-course of the experiment.
- 3 **Third**, it may be possible to examine unstable complexes at **low temperatures**, when the rate of decomposition of such complexes will be decreased.



Chemical Modification of Side-Chains

The principle underlying the application of chemical modification techniques to the study of enzyme mechanisms is **very simple**.

If an amino-acid side chain involved in the catalytic activity is **chemically modified**, the enzyme will be **inactivated**.



Site-Directed Mutagenesis



An invaluable tool

Site-directed mutagenesis is an invaluable tool to **modify genes** and study the structural and functional properties of a protein, based on the structure, function, **catalytic mechanism**, and **catalytic residues** of enzymes.

The development of **recombinant-DNA technology** has led to the possibility of introducing **amino-acid replacements** at specified positions in a protein.

Site-directed mutagenesis is a molecular biology method that is used to make **specific and intentional changes** to the DNA sequence of a gene and any gene products (proteins).

THIS WAS POSSIBLE IN VIEW OF THE FACT THAT



Gene available

The gene coding for the protein is available.



Expression system

There is a suitable system for expression of the gene.

THE FIVE COMPLEMENTARY EXPERIMENTAL APPROACHES

1

Kinetic studies

2

**Detection of
intermediates**

3

**X-ray
crystallography**

4

**Chemical
modification of
side chains**

5

**Site-directed
mutagenesis**



To Summarise...

MECHANISM 3

Deducing a mechanism

- Sequence of enzyme-containing complexes
- Rates of interconversion
- Structures of these complexes

ENERGY ΔG

Energy profile

- An energy barrier (ΔG) must be surmounted
- Enzymes reduce the free energy of activation
- Stabilization of the transition state is the mechanism

CATALYSIS 4

Rate-enhancing factors

- Proximity & orientation effects
- Acid-base and covalent catalysis
- Changes in environment; strain or distortion

APPROACHES 5

Experimental approaches

- Kinetic studies; detection of intermediates
- X-ray crystallography; chemical modification
- Site-directed mutagenesis — complementary

TS ‡

Transition state

- Highest point(s) on the reaction coordinate
- Lifetime no longer than a vibration
- Cannot be isolated; one per step

I I

Intermediate

- Comparatively long-lived; can be detected
- Relatively stable midpoint (valley)
- A reaction doesn't have to have one

READING AN ENERGY DIAGRAM

Beginning = reactants



Hilltops = transition states



Valleys = intermediates



End = products