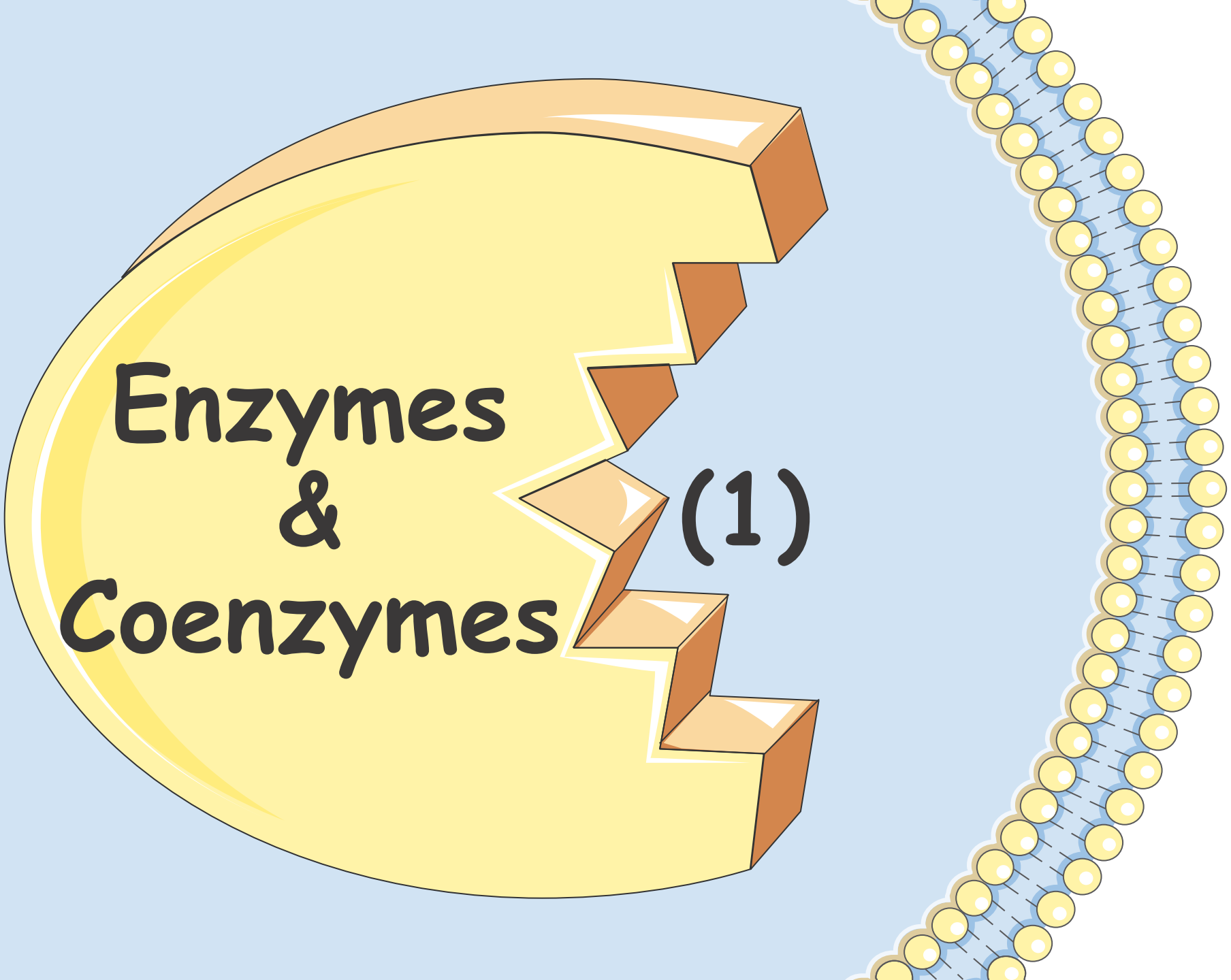
Enzymes & Coenzymes (1)



MED441
KING SAUD UNIVERSITY

Revised & Reviewed by: Abdulaziz Bahammam & Faye Wael Sendi

7
V1

Foundation Block - KSU

Color Index:

- Main text
- Important
- Notes
- Boys slides'
- Girls slides'
- Extra

Editing File

Objectives

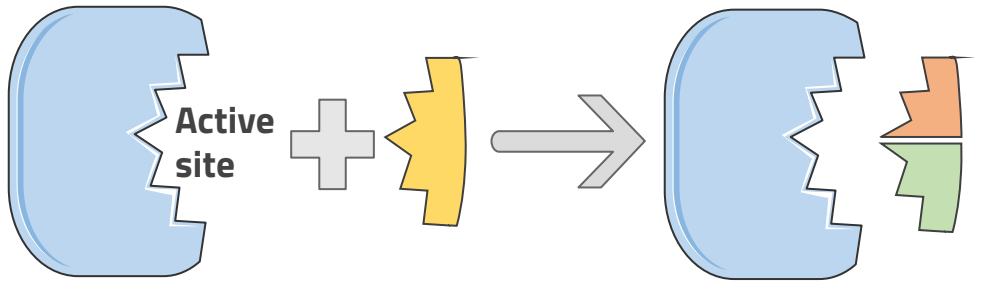
- Understand how enzymes are able to speed up the rate of biochemical reactions in the body.
- Identify classes of enzymes based on the type of reactions they catalyze.
- Comprehend basic terms of: coenzymes, isoenzymes, enzyme activity & specificity along with factors affecting their activity.
- Understand the enzyme kinetics.

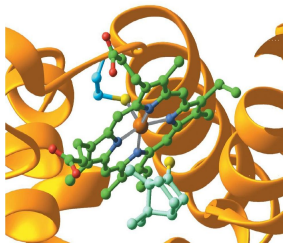
Enzymes

helpful video

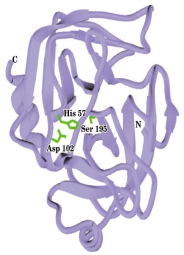
"**Biological catalysts** (محفزات) that speed up the rate of a reaction **without being consumed**/changed in the reaction"

Structure	all enzymes are **protein** in nature, but NOT all proteins are enzymes (others are structural, transporters, etc.) Exception: ribozymes are RNA (ribonucleic acid) molecules with enzymatic activity		
Function	bind to specific **substrate** "substance upon which enzymes act" (like a reactant) to convert them to **product(s)**		
Properties	**1) Active site**	▪ all enzymes have ≥1 **active sites** : "regions where substrate binds" (catalysis occurs once they bind)	
	2) Specificity	enzymes are **highly specific**	▪ they interact with only **1 or few** substrates if they have very similar structures ▪ they catalyze only **1 type** of reaction even if it's the same substrate (437)
	3) Regulation	enzymes can be activated or inhibited so that the **rate of product formation** responds to the **need of the cell** ▪ some enzymes also have **regulatory sites** that control the active site in different ways (next lecture's topic)	

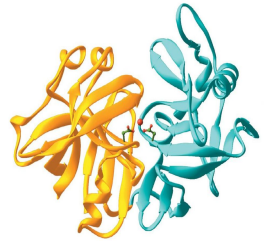




an **enzyme** with its active site



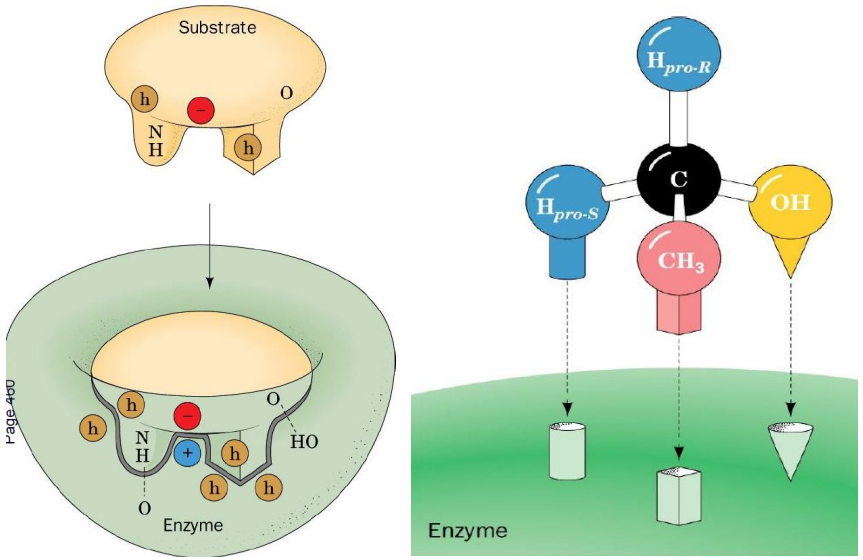
Trypsin (a digestive enzyme in small intestine)

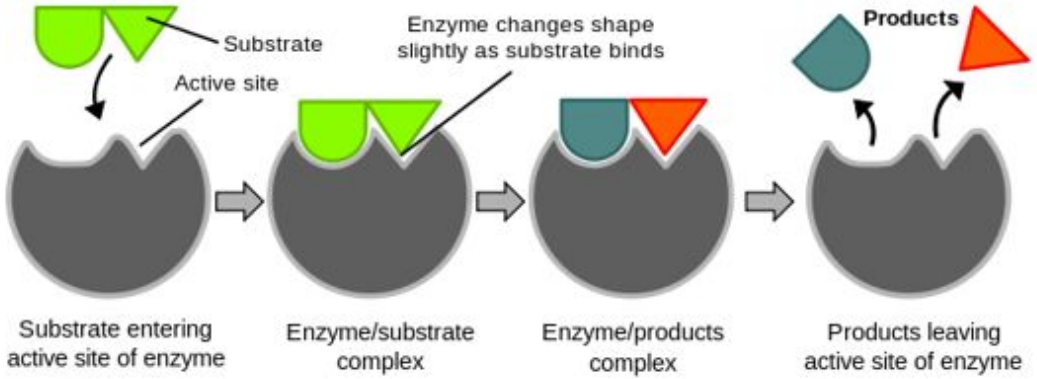


Pepsin (a digestive enzyme in stomach)

Models of enzyme-substrate binding

Lock & key binding	Induced-fit binding
Active site fits the **exact dimensions** of substrate **[exactly complementary]** زي القفل ما يفتحه إلا مفتاح واحد نفس شكله بالضبط	After the binding of substrate, the **enzyme changes its shape** (conformation) to fit more perfectly with substrate **[not fully complementary,** after the reaction is done, it goes back to its original shape to be able to function again**]** زي القفاز ياخذ شكل اليد بعد ما ينلبس





Classification of enzymes

helpful video

Classified into 6 types according to the type of reaction catalyzed:

(436) Omar Tried Hard Learning International Languages

Class	Type of reaction (439)
1. Oxidoreductases	Oxidation-reduction reaction
2. Transferases	Transfer of functional groups
3. Hydrolases	Hydrolysis (breaking bonds by adding water)
4. Lyases	Group elimination to form double/triple bonds
5. Isomerases	Isomerization (form isomers)
6. Ligases	Bond formation coupled with ATP hydrolysis

Nomenclature (naming)

	Common name	Systematic name
Rule	Suffix **"-ase"**	based on the rules given by **IUBMB** (International Union of Biochemistry & Molecular Biology): **EC Class . Subclass . Subsubclass . Enzyme number** (**EC** = Enzyme Commission)
Example	(E.g. amylase) Exceptions: pepsin, trypsin	Example: **EC 3.4.17.1** (carboxypeptidase A) Hydrolase, breaks a bond Which bond? peptide (439) Which enzyme exactly? hypothetically #1 Which peptide bond? alanine & serine for ex. *This is just an example, don't memorize it

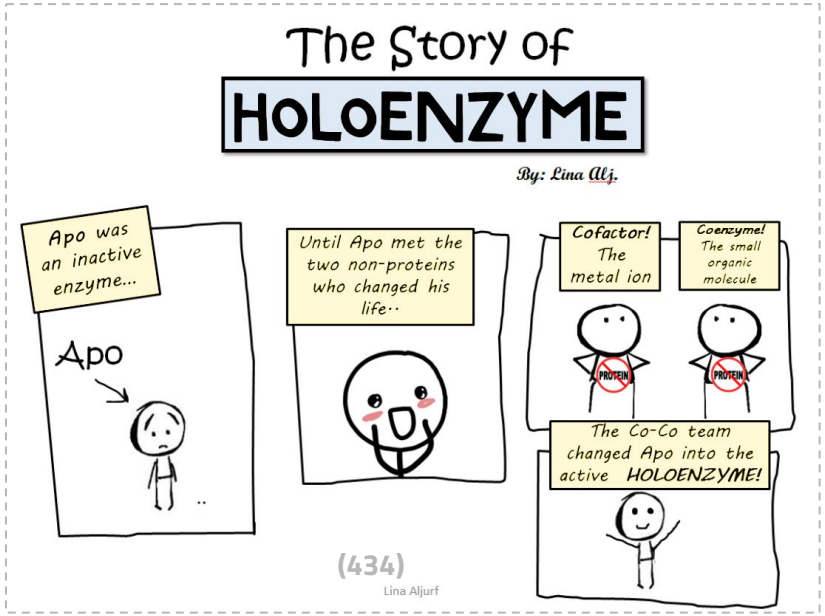
Basic terms

Some enzymes require **non-protein** groups to catalyze a reaction (become active):

Apoenzyme	+	Non-protein part	=	Holoenzyme
inactive form of enzyme **without its non-protein part**		required by some enzymes to become active; help to catalyze a reaction		**active** complex

- **Coenzyme**: small **organic** molecules (E.g. NAD^+, FAD, vitamins)
  - **Prosthetic Group**: **permanently** associated with enzyme (E.g. **FAD**) always attached
  - **Cosubstrate**: **temporarily**/transiently associate with enzyme (E.g. **NAD**) only attached during the reaction
- **Cofactor**: **metal** ion (E.g. Cu^{2+}, Fe^{3+}, Zn^{2+})

- The majority of enzyme require these molecules
- They are present in the active site as an additional molecule



Ribozymes: **RNAs** (ribonucleic acid) with enzymatic activity (they're the only non-protein enzymes)

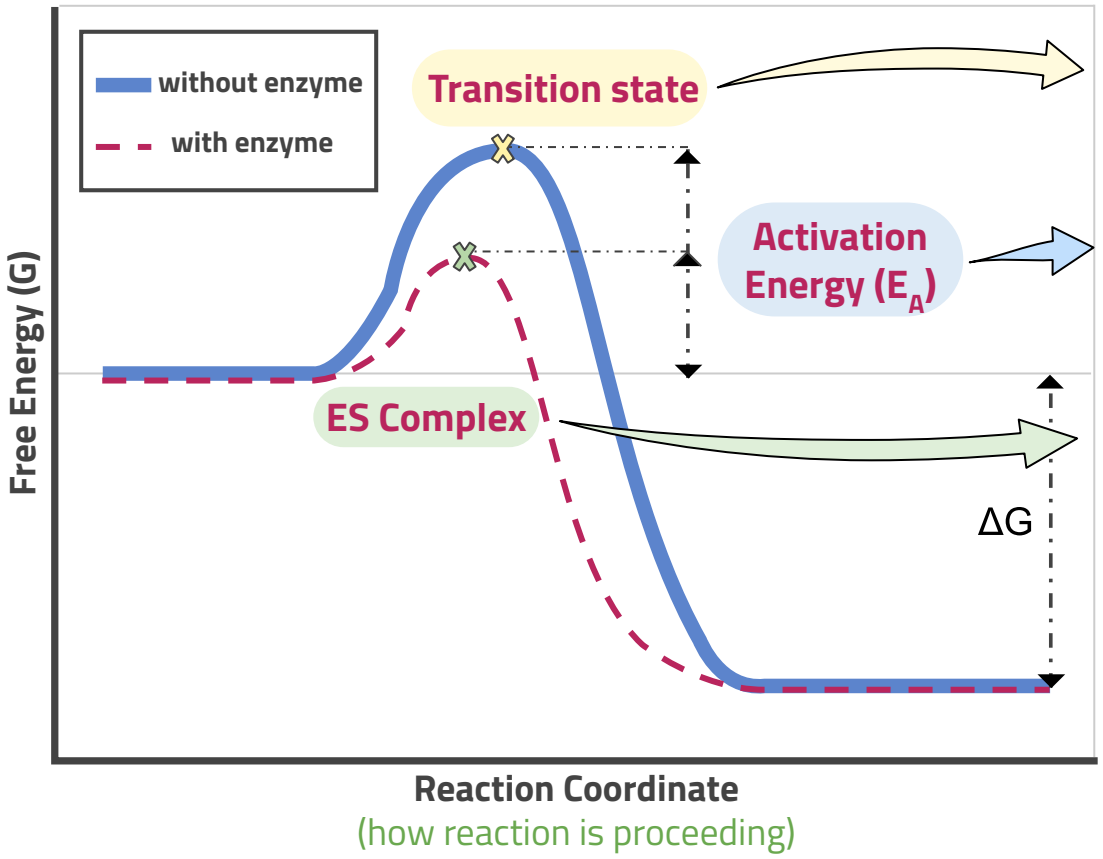
Isoenzymes: enzymes that catalyze the **same chemical reaction** but have slightly **different structures**
They're usually found in different locations [Ex. blood, tissue]

Zymogens: **inactive** enzyme precursors that require a biochemical change to become active
(E.g. cleavage of a peptide blocking the active site, phosphorylation). They're activated when needed
This provides a way of regulation. For example, [pepsinogen → pepsin] only when we're eating

(Aka **proenzymes**)

(434): notice that zymogens & apoenzymes need different things to become active:
Apoenzymes require a coenzyme/cofactor
Zymogens require a biochemical change

How enzymes work



It is a **high-energy** intermediate that reactants pass through in every chemical reaction; it has **greater energy** that than that of reactants or products alone

It is the **difference in energy** between **reactants** & **transition state**
- If activation energy is available, the reaction can proceed forming products
- Uncatalyzed reactions are often slow due to high E_A

In order for enzymes to speed up reactions, they must:
1) **decrease the activation energy** required for a reaction, providing an **alternative transition state** of **lower energy** called **enzyme-substrate complex** and thus speed up the reaction.
2) **NOT alter the change in the free energy (ΔG)** "energy that can do work"
Why? because we don't want them to change the equilibrium of the reaction; rather, we need them to accelerate the rate by which equilibrium is reached, speeding up both forward & reverse rates

Summary of how enzymes work:
1) Activation energy is reduced
2) Free energy remains the same

(436)
Enzyme induction = increasing activity
Enzyme inhibition = decreasing activity

ΔG of products must be less than ΔG of reactants

Enzyme activity or velocity

Velocity: the rate of a reaction catalyzed by an enzyme
- **Enzyme activity** is expressed as **μ moles** of product formed/**min**/**mg** enzyme

Enzyme velocity can be measured by either:
- how much product is increased / formed (more common)
- how much substrate conc is decreased

Factors that affect enzyme activity

1) Temperature

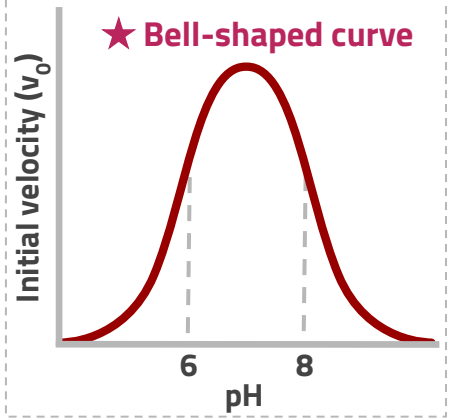
Every enzyme has an **optimal temp** for catalyzing a reaction → in **humans** most enzymes have an optimal temp of **37°C**
- The **rate** of an enzyme reaction **initially increases** with rise in temperature (increase in velocity → until it reaches **peak** velocity (very **active**)
- But at **high** temp [E.g. above 40°C] enzymes are **denatured** → become **inactive**

2) pH

Every enzyme has an **optimal pH** for catalyzing a reaction, which is the peak of a bell-shaped curve
↳ most have highest activity between **pH 6-8** [**Exception:** pepsin has highest activity at **pH 2** in the stomach]
- pH affects **catalysis** through either the **substrate** or **ionizable groups** in the active site of enzyme

Extra: Ionizable groups are those that are able to gain/lose H^+, such as COOH & NH_2 in amino acids. Catalysis requires both substrate & active site to have a specific chemical group in either ionized or unionized states, which is directly affected by pH

Effect of **pH** on the initial rate of the reactions catalyzed by **most enzymes**



3) [S] & [E] concentration

*Square brackets [ ] depict concentration

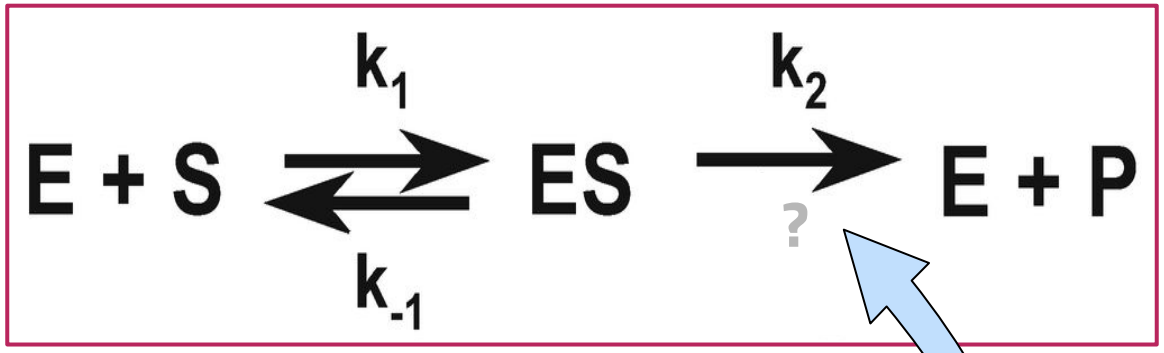
At **low [S]**
- the reaction velocity/rate (v) is **proportional to [S]** (increases initially with increasing [S])

at **high [S]**
- further addition of **substrate** has **no effect** on enzyme velocity because enzyme is saturated & all active sites are engaged
- the rate of an enzyme reaction is directly **proportional to [E]** if [S] is higher than [E]

For example, if there are 50 enzyme molecules & 70 substrates, only 50 substrates will be catalyzed as we have a limited number of active sites

Enzyme Kinetics

Basic reaction model



In this reaction model, ES complex has 2 fates:
1) Continue to form product [right]
2) Go backwards [left]

S = Substrate
E = Enzyme
ES = Enzyme-Substrate complex
P = Product
k_1, k_{-1}, k_2 = Rate constants

Extra:
Why is k_{-2} missing? Although step 2 is reversible, we do not consider its reverse reaction because our study of kinetics is during the early phase, where there is a very low concentration of product, so k_{-2} is negligible

Initial rate of enzyme reaction

helpful video

1) Pre-steady state kinetics

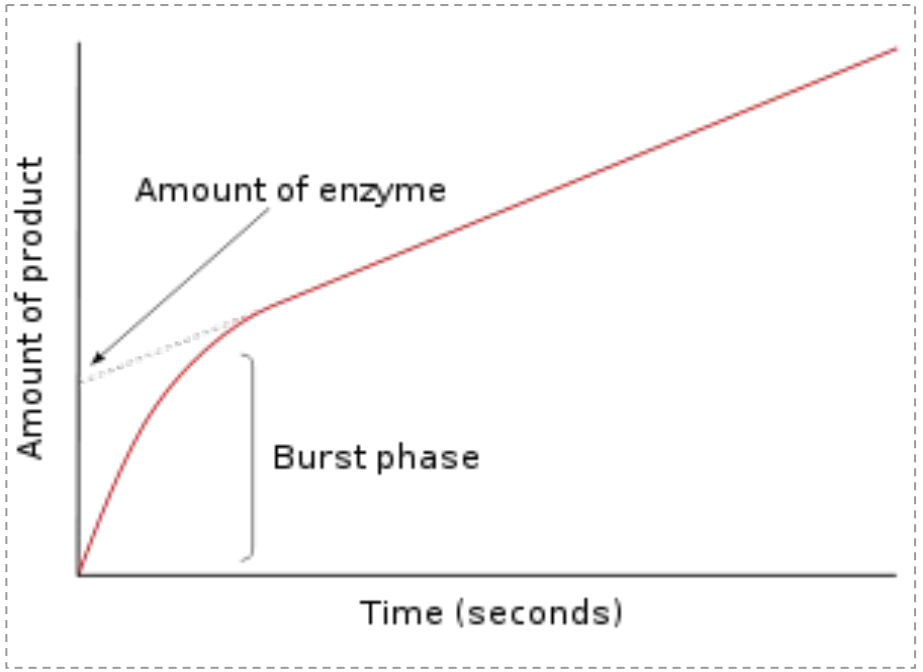
When an enzyme is mixed with high [S], there is an **initial** short period of time (a few 100 microseconds) during which **intermediates** leading to the formation of product **(ES) gradually build up** (no product has formed yet)

أول ما نخلط الإنزيم مع الـsubstrate يبدون يرتبطون ببعض ويكونون الـ ES، واللي وهو لسا ما يعتبر ناتج تفاعل (product)

2) Steady state kinetics

After initial state, the **reaction rate** and **concentration** of intermediates change **slowly** with time, and the intermediate is said to be in **steady state** because its **rate of synthesis is equal to its rate of degradation**
rate of formation of ES = rate of breakdown of ES to (E+S) & (E+P)

بعدها يثبت تركيز الـES complex لأنه ينبني ويتحطم بنفس المعدل (نلاحظ ثبات الميل)، وهنا يتكون الـproduct

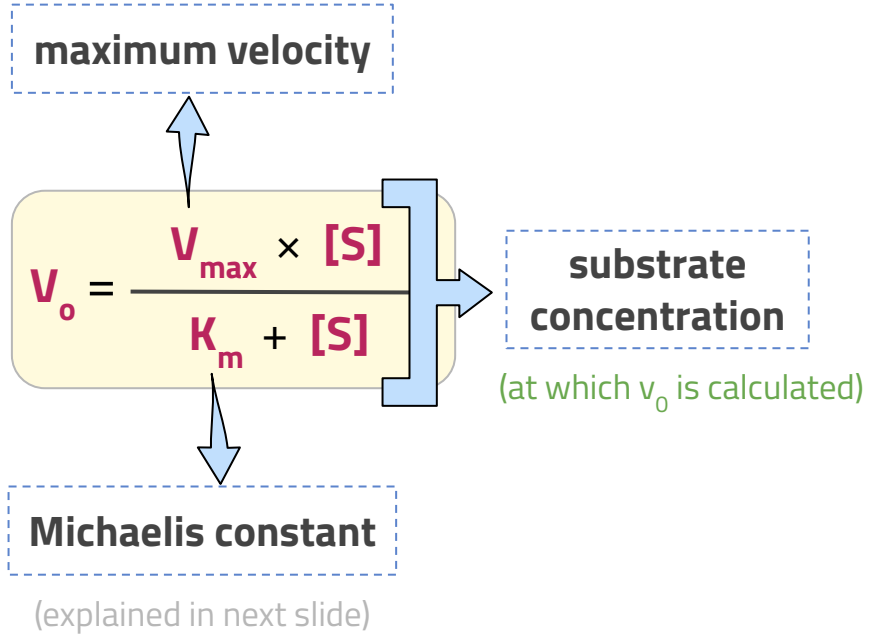


Summary		
	Pre-steady state	▪ ES builds up ▪ No product yet
	Steady state	▪ ES is constant ▪ Product forms

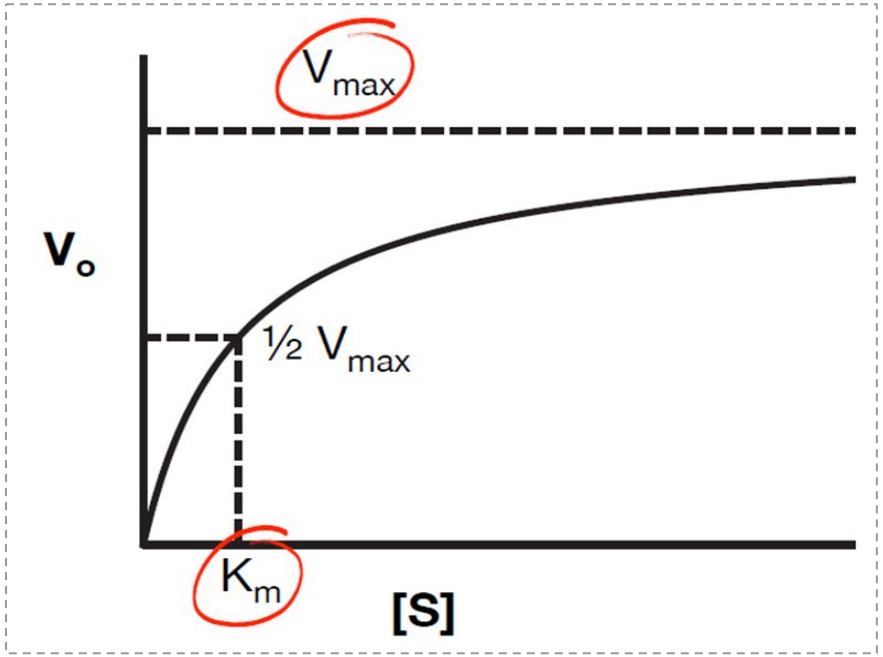
Michaelis-Menten

- The model of enzyme kinetics was first proposed by **Michaelis & Menten** in 1913 and **later modified** by Briggs & Haldane
- The **Michaelis-Menten equation** describes the relationship of **initial rate** of an enzyme reaction to the **[S]**
- It measures the **initial velocity (V_o)** of an enzyme reaction

❖ Equation



❖ Graph



K_m (Michaelis Constant)

Quantitatively (numerically):

- It is the substrate concentration at which the **initial rate is one-half of the maximum rate (½ V_{max})**
- It is the substrate concentration **required to saturate half of all of the active sites of an enzyme**

Qualitatively (how it benefits us or what it indicates):

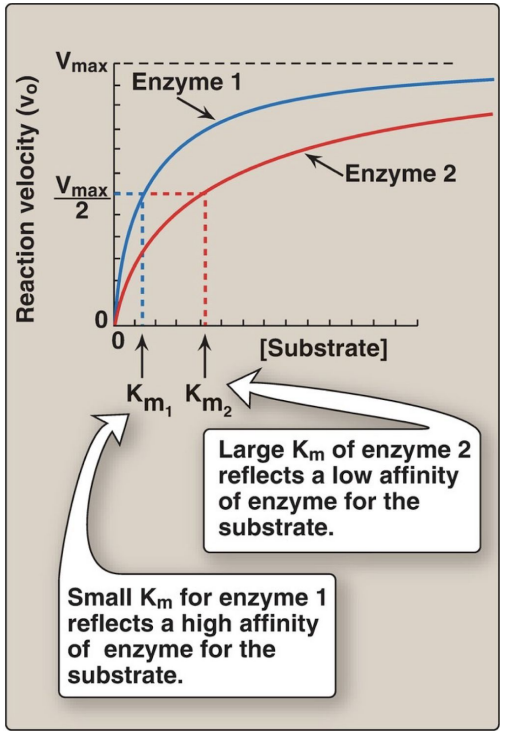
The K_m value of a substrate depends on its **affinity** with an enzyme

High K_m

means **low affinity** with enzyme (**more substrate** is needed to saturate the enzyme)

Low K_m

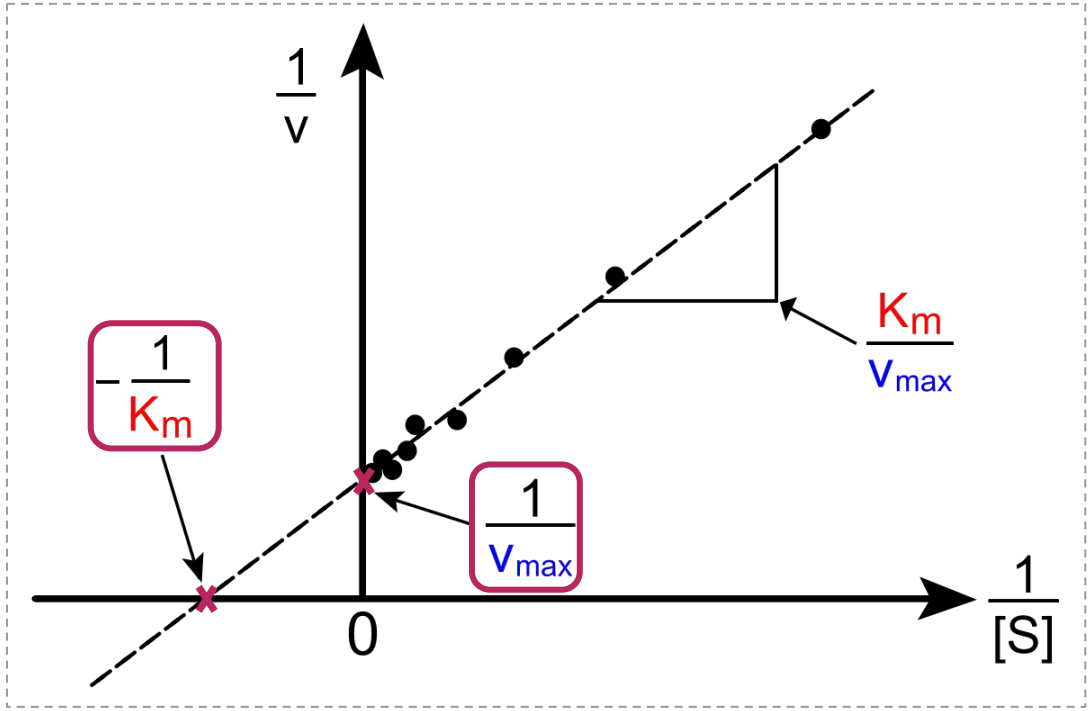
means **high affinity** with enzyme (**less substrate** is needed to saturate the enzyme)



An **extra graph** to understand it

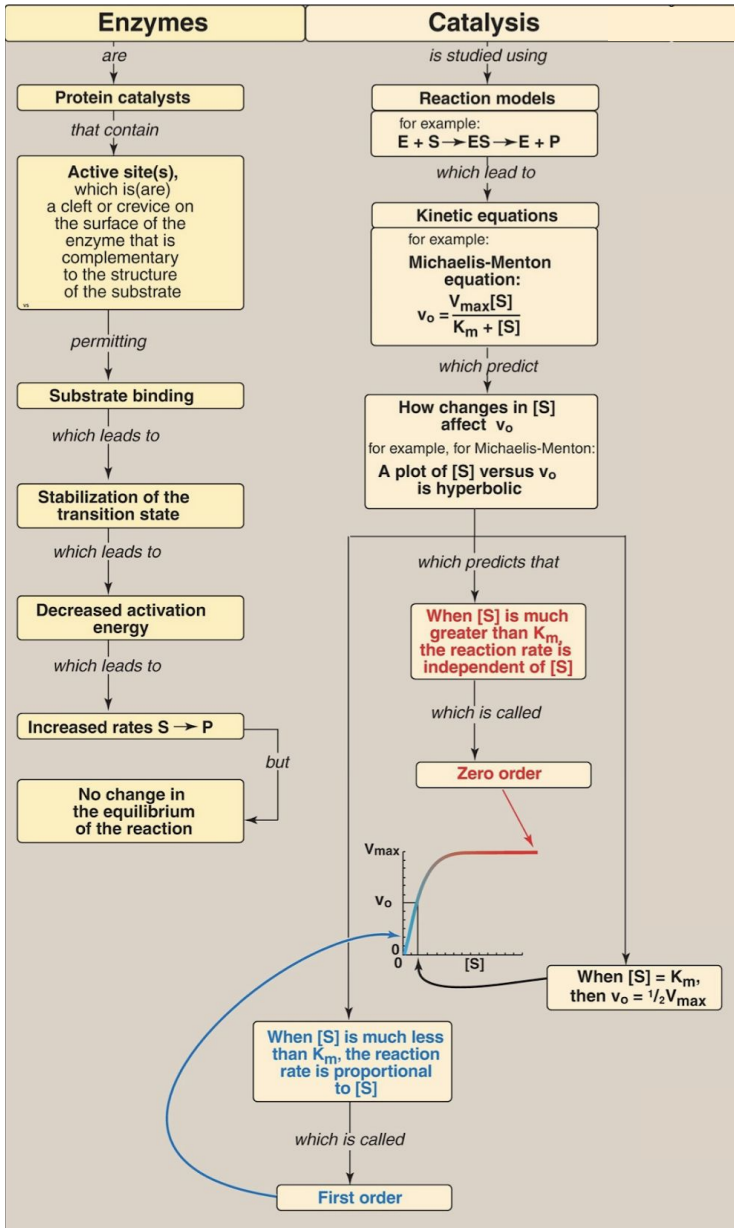
Lineweaver-Burk

- **Also called:** double-reciprocal plot
- **It's obtained from:**
  taking reciprocals (مقلوب) of the **Michaelis Menten equation**
- **It's plotted to:**
  - calculate the K_m and V_{max} values more accurately
  - determine the mechanism of action of enzyme inhibitors



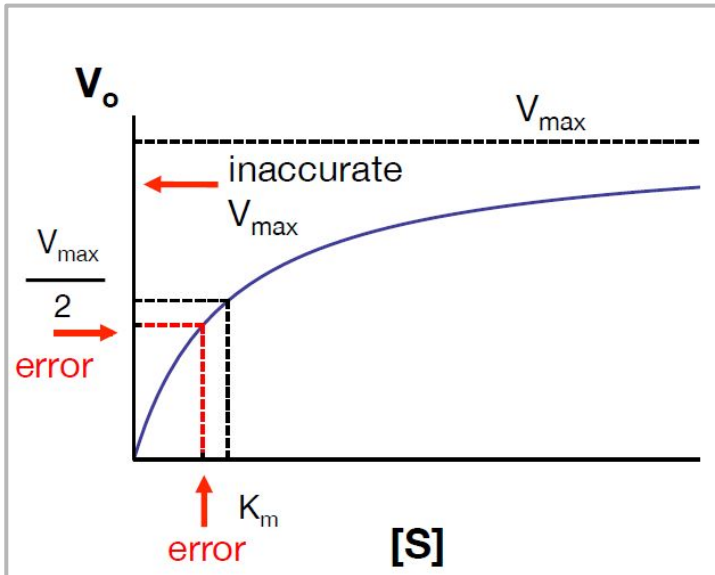
How is it different than the Michaelis-Menten plot?
The Michaelis-Menten plot is hyperbolic (curved), which makes it inaccurate as it gives multiple values for v_{max}. Thus, Lineweaver & Burk suggested to linearize it (graph the same data as a straight line) to improve accuracy.

Summary

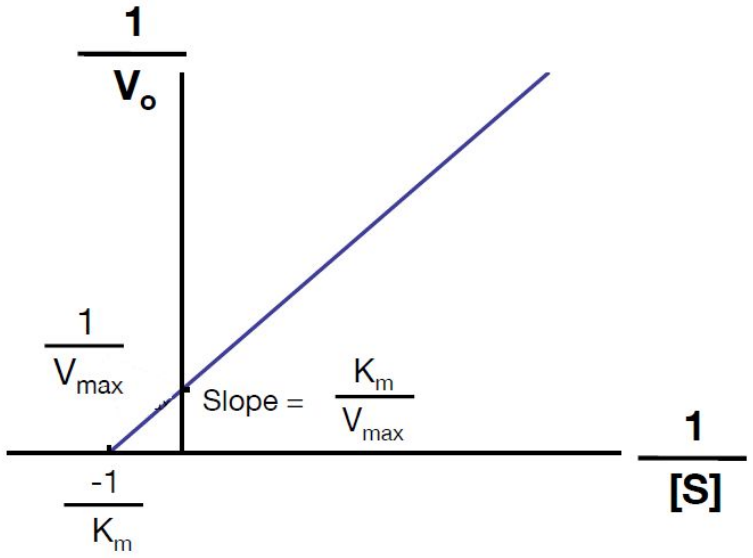


Side-by side comparison

Michaelis-Menten



Lineweaver-Burk



less error when determining Km

Quiz

Q1: Which class of enzymes requires ATP hydrolysis to function?

A	Hydrolases	B	Ligases	C	Lyases	D	Transferases

Q2: Which is true about isoenzymes?

A	They catalyze the same reaction	B	They have the same structure	C	They react with the same substrate	D	A & C

Q3: When enzymes decrease the activation energy, the free energy (Δ G) is:

A	Increased	B	Decreased	C	Unchanged	D	It depends

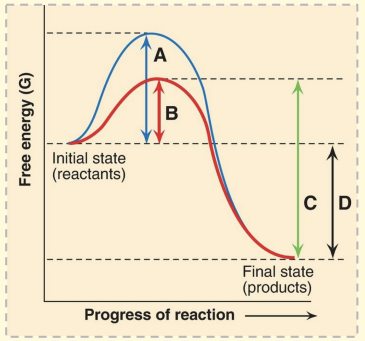
Q4: Which of the factors affecting enzyme activity follows a bell-shaped curve?

A	pH	B	temperature	C	[E]	D	[S]

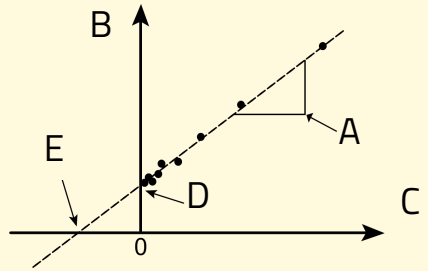
Q5: High K_m value indicates:

A	Low affinity with enzymes	B	High affinity with enzymes	C	Initial velocity equals zero	D	Steady state reaction

Answer Key: 1) B 2) D 3) C 4) A 5) A



Q6: Which letter represents the free energy of the reaction?

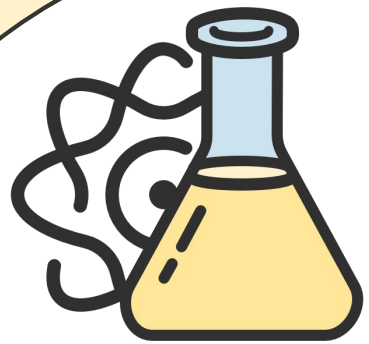


Q7: What does the letter D represent in the Lineweaver-Burk plot above?

Answers

Q6: D

Q7: V_{max} inverse ($1/V_{max}$)



Biochemistry 441

Girls

Boys

Ghadah Alarify - Leader

Yara Almufleh
Reema Alrashedi
Wareef Almousa
Joud Alangari
Fay Alluhaidan
Sarah Alhamlan
Arwa Almobeirek
Jumana AL-qahtani
Latifa Alkhdiri
Alanoud Alhaider
Futoon Almotairi
Manal Aldhirgham
Raaoum Jabor
Norah alawlah
Shahad Helmi
Rand Aldajani

Khalid Alhamdi - Leader

Ahmed Alayban
Sultan Alosaimi
Abdullah Alomran
Bassam Alghizzi
Ibrahim Aljurayyan
Mohammed Almutairi
Turki Alkhalifa
Malik Alshaya
Faisal Alhmoud
Abdulrahman Alnoshan
Ahmed Alqahtani
Hamad Alshaalan
Anas Alharbi
Mohammed Alwahibi
Saad Alghadir
Firas Alqahtani

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