Enzymes and Kinetics

Knut Klokk

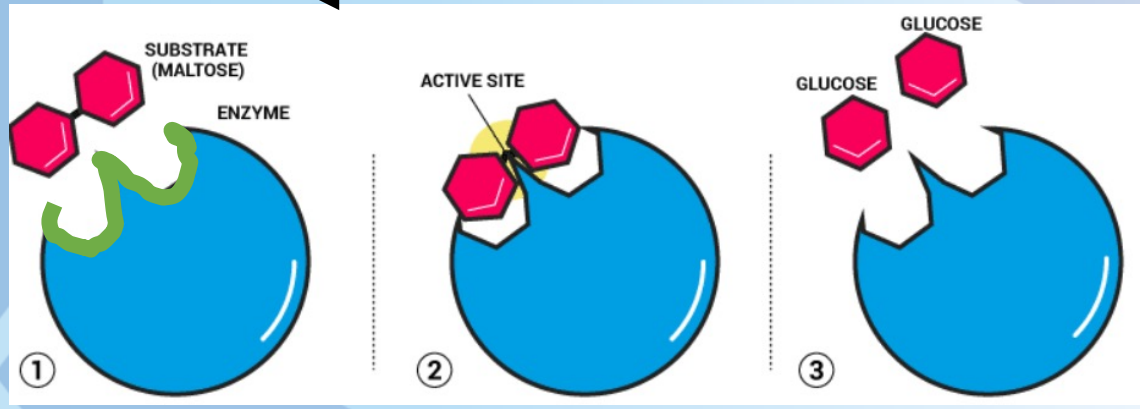
Contents!

1. Introduction to Enzymes

General info:

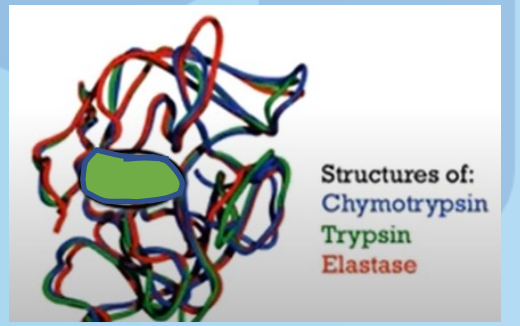
-Enzymes are biocatalysts

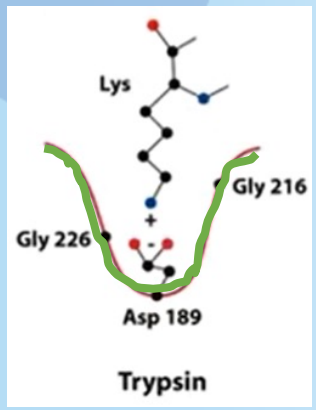
-Catalysts are substances which ↑ the rate of a reaction **without being changed in the process**

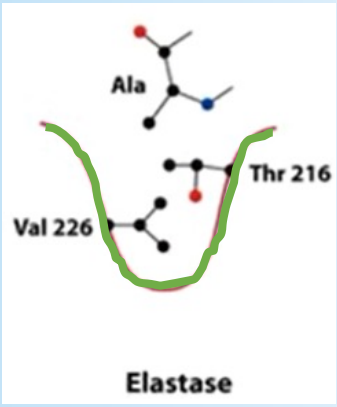


-Most enzymes are **proteins**(tertiary/quaternary)
But not all: Ribozymes (RNAs with catalytic activity)

-**Active Site** =
Where catalysis takes place
Highly specific to its substrate(s)
Different amino acids means
- Control of environment
- 3-D Pocket dimensions
- Sometimes multiple interactions with substrate







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Some enzymes require cofactors to function!

Holoenzyme = **Apoenzyme** + **???**

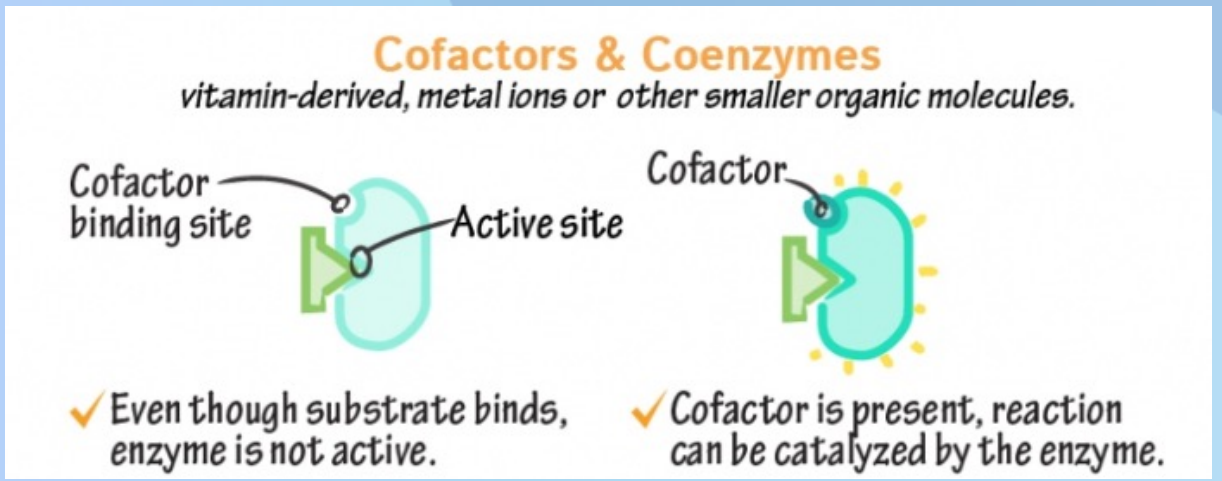
"Whole" enzyme ☺ | "A-protein" | **Non-protein**

Inorganic Molecule "Cofactor"
(Metal ions)
ex: Zinc, Iron, Copper, Calcium, Magnesium, Potassium, ect)

Organic Molecule "Coenzyme"
(Usually vitamins)

1. Prosthetic group (Tightly bound)
-Returned to original form
-ex: FAD

2. Co-substrate (loosely bound)
-Return in an altered state
-ex: NAD



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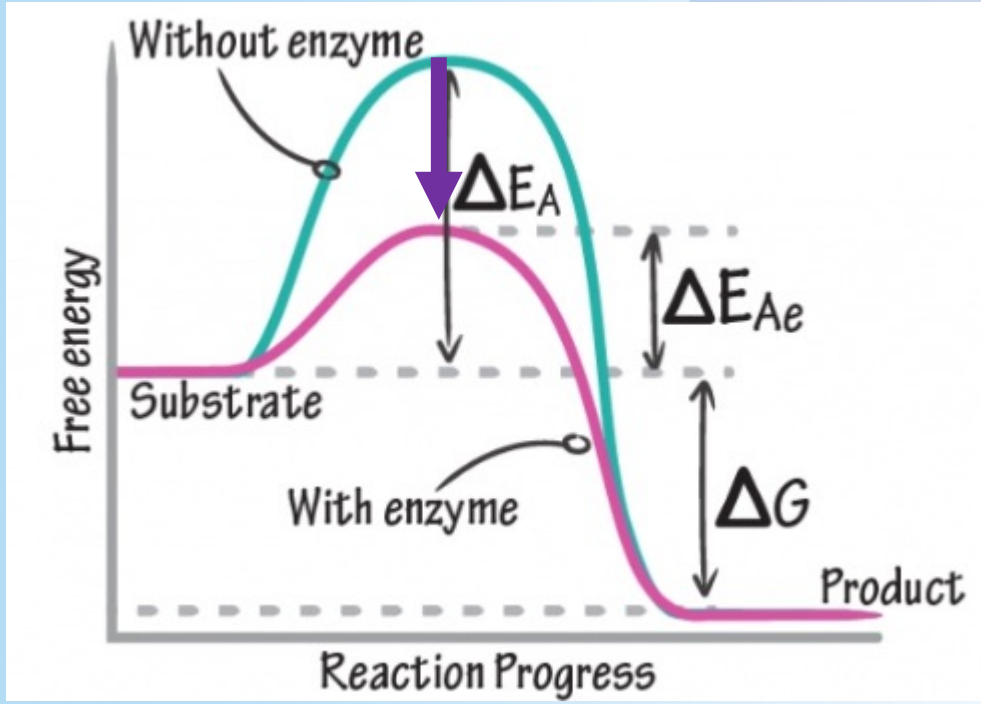
Contents!

3. Energy changes = Enzymes lower the activation energy ($\downarrow E_A$)

$$E + S \rightleftharpoons ES \rightarrow E + P$$

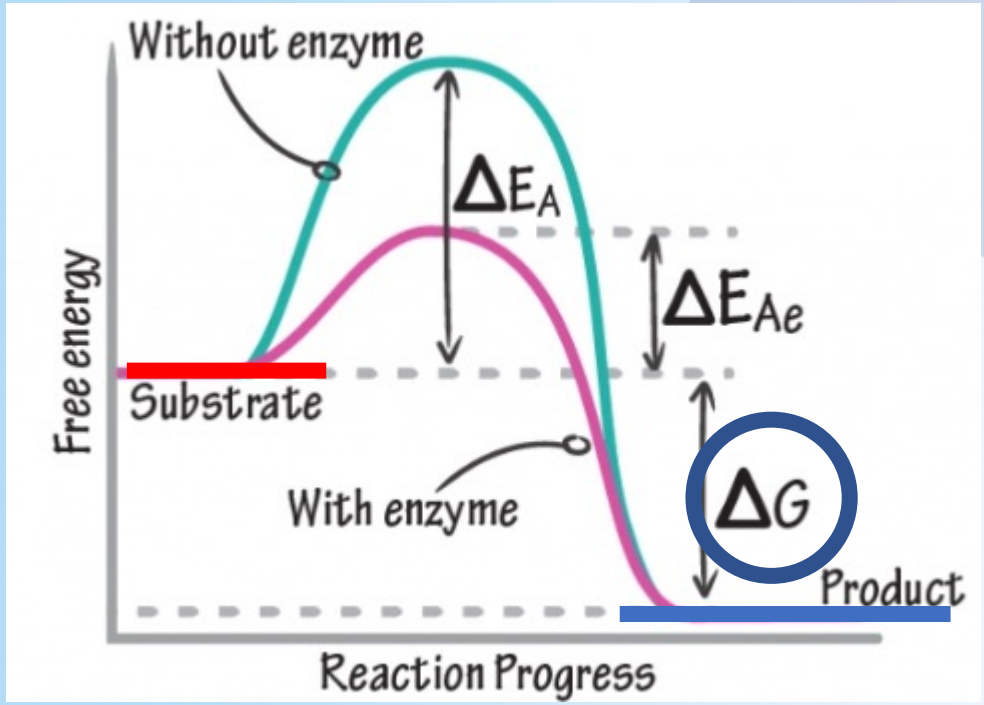
-↑ Rate of reaction, by ↓the ACTIVATION energy
-EA = The energy barrier required to overcome for a reaction to proceed
(energy needed to make reaction happen)

-How?
By stabilizing the transition state between the substrate and product, provided by an alternate mechanism with lower EA



3. Energy changes = Enzymes lower the activation energy ($\downarrow E_A$)

$$E + S \rightleftharpoons ES \rightarrow E + P$$



-Catalysts are substances which ↑ the rate of a reaction **without itself being changed in the process, by lowering the activation energy.**

There is NO difference in the Gibbs FREE Energy of the overall reaction! (ΔG)
-The same initial substrates and final products
-"The energy difference between substrate and product"

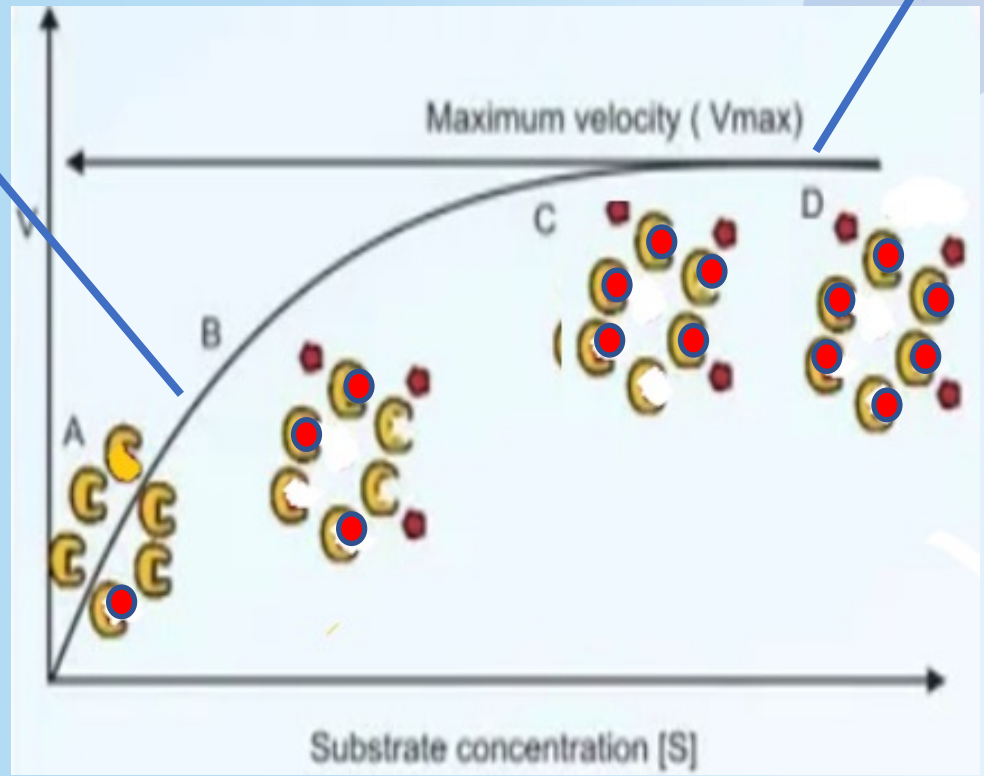
When (ΔG) is:
-Negative = Favors the forward reaction
-Positive = Favors the reversal reaction

4. Factors which affect the reaction velocity

Velocity of reaction = $\frac{\text{Mol}}{\text{Unit time}}$

First Order
Rate = k [A]^1

Zero Order
Rate = k[A]^0



A. Substrate concentration

-V will ↑ with ↑ Substrate until saturation = Vmax
-First and Zero order kinetics

Saturation = ↑ substrate concentration

B. Temperature

C. pH

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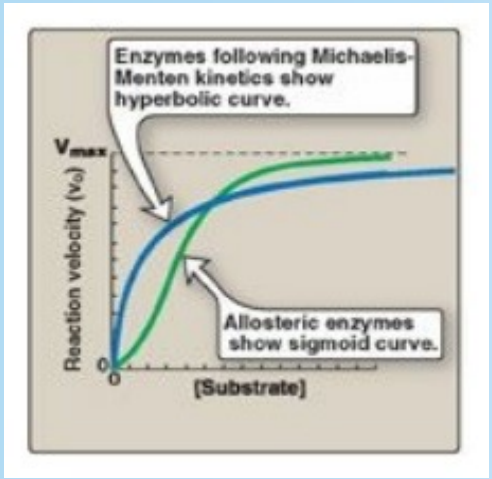
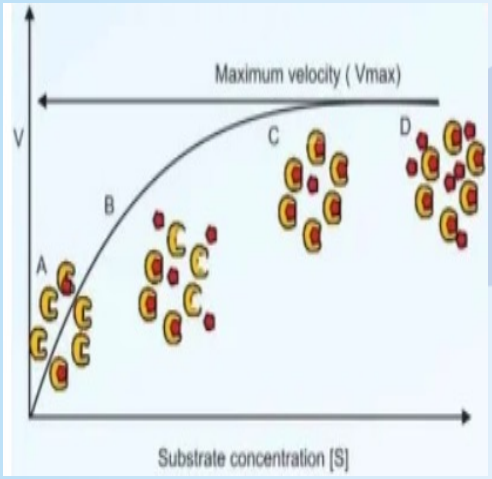
4. Factors which affect the reaction velocity

$$\text{Velocity of rx} = \frac{\text{Mol}}{\text{Unit time}}$$

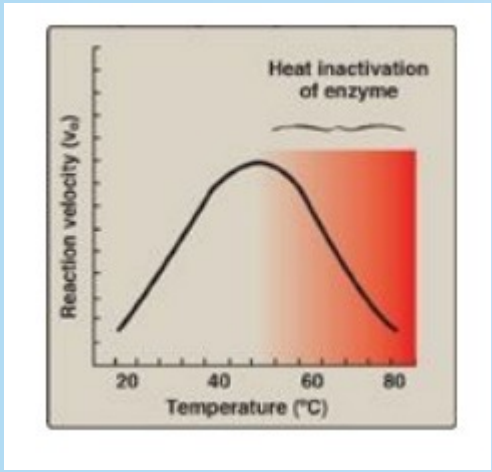


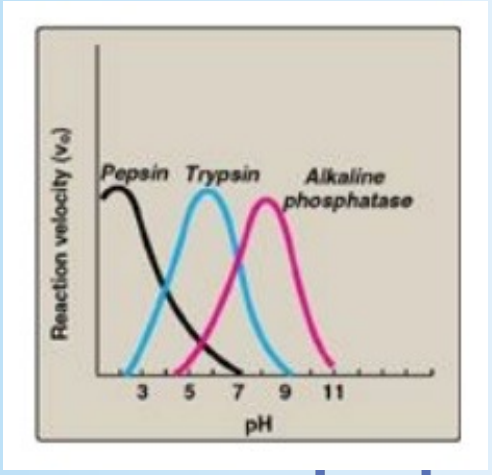


A. Substrate concentration

-V will ↑ with ↑ Substrate until saturation = Vmax

-First and zero order kinetics





B. Temperature

-V will increase with increased temp until **denaturation**

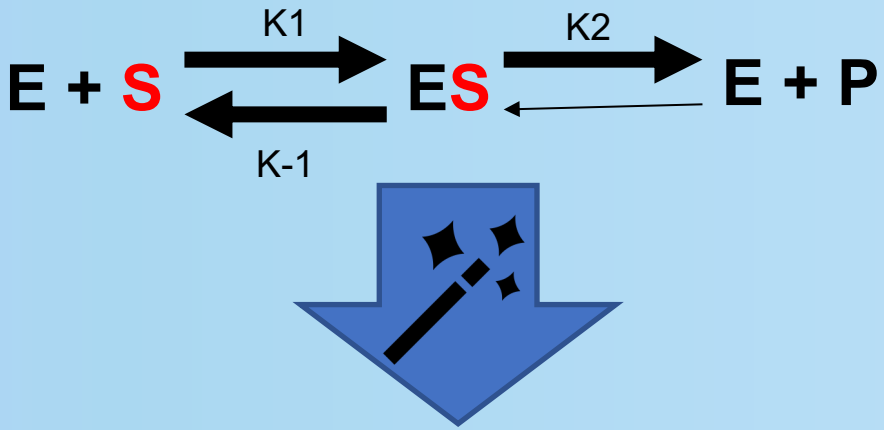
C. pH

-Some enzymes need to be ionized or unionized to work

-**Denaturation** may occur at certain pH

Contents!

5-Michaelis-Menten



K1, K2,K-1 = Rate constants

Vmax = Maximal velocity

Vo = Initial reaction velocity

$$V_o = \frac{V_{max}[S]}{K_m + [S]}$$

[S] = Substrate concentration

Km = Michaelis constant

5-Michaelis-Menten

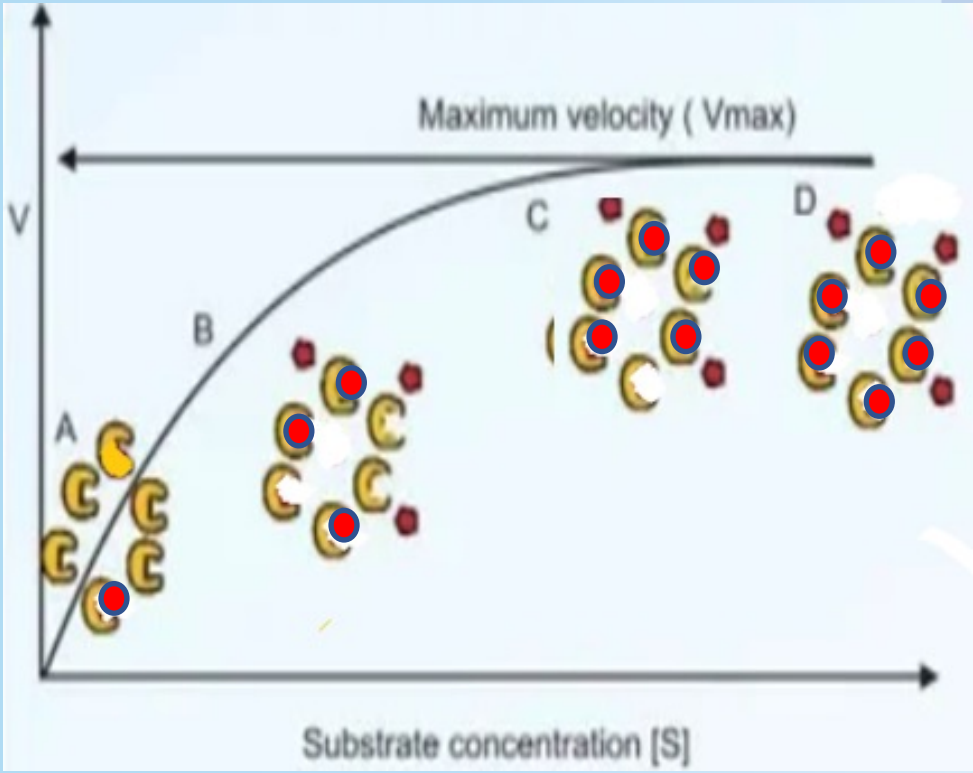
$$V_o = \frac{V_{max}[S]}{K_m + [S]}$$

$$E + S \underset{K-1}{\overset{K1}{\rightleftharpoons}} ES \overset{K2}{\rightarrow} E + P$$

Vmax = Maximal Velocity

"As the [S] keeps increasing, we end up with a steady state in which all the enzyme is bound. At this point, we have reached maximum velocity"
-Enzyme is fully saturated by substrate at Vmax and working at its maximum velocity!
-All active sites are filled!

-Vmax = Mol/Unit time



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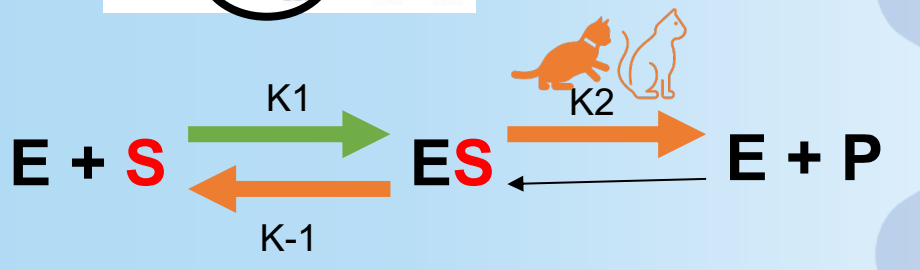
↑Km = ↓Affinity
↓Km = ↑Affinity

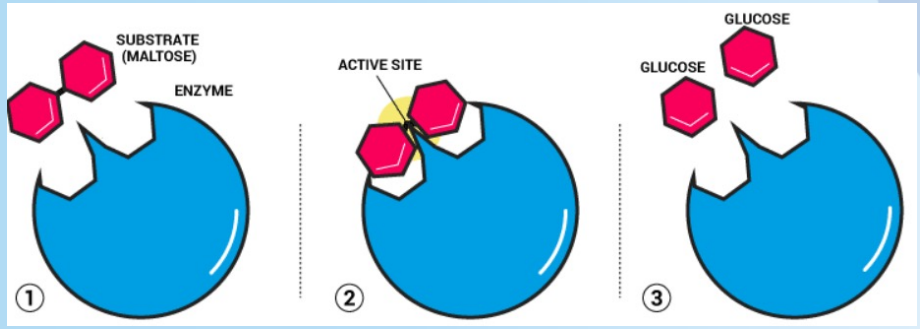
5-Michaelis-Menten

$$V_0 = \frac{V_{max}[S]}{K_m + [S]}$$

K1, K2,K-1 = Rate constants
K2 = Kcat = "Rate limiting step"
"Turnover number"
= Vmax / [E]Total
Relates the Speed of catalysis
-Basically how much substrate 1 enzyme can convert in 1 second.

$$V_0 = \frac{k_{cat}[E_{tot}][S]}{K_m + [S]}$$





Km = Michaelis Menten Constant

-Shows affinity of an enzyme to its specific substrate

$$Km = \frac{K\text{-}1 + K2}{K1}$$

K2 = Dissociation
K1 = Formation

-As we ↑Km the "affinity" (binding) to the substrate will ↓

-As we ↓Km the "affinity" (binding) to the substrate will ↑

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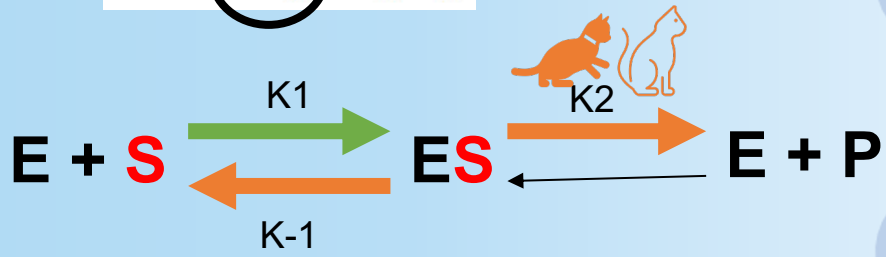
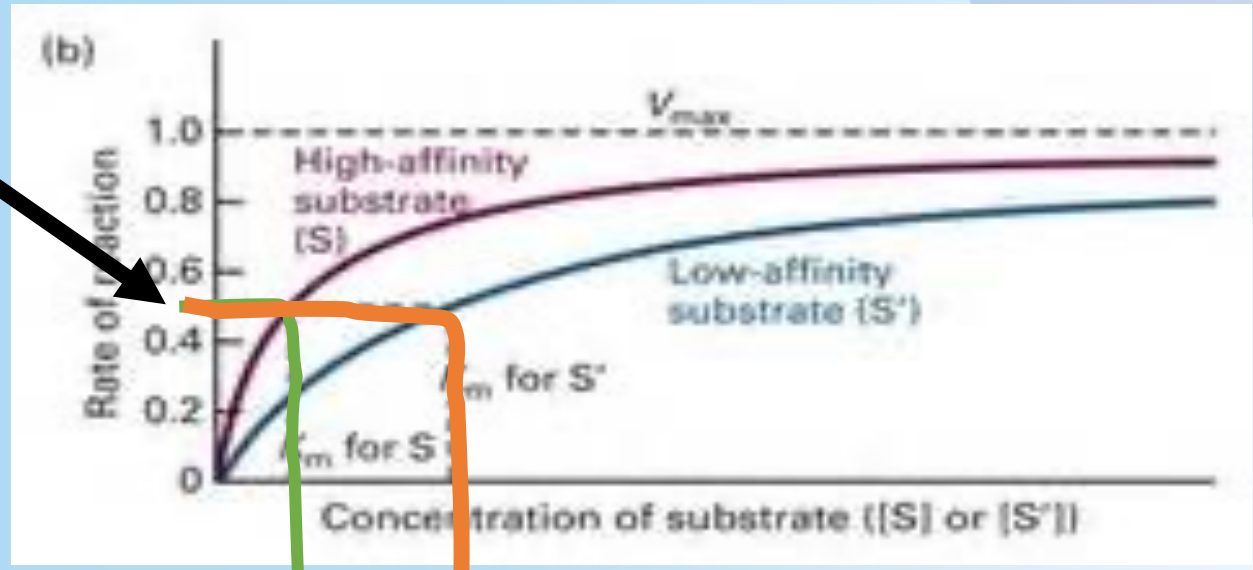
↑Km = ↓Affinity
↓Km = ↑Affinity

5-Michaelis-Menten

$$V_o = \frac{V_{max}[S]}{K_m + [S]}$$



Km = Michaelis Menten Constant
- Shows affinity of an enzyme to its specific substrate
- Substrate concentration at which Vo is exactly ½ Vmax



~~Km = ½ Vmax~~

Small Km = low [s] needed to reach ½ Vmax
(because of the strong affinity)

Large Km = Lots of [s] needed to reach ½ Vmax
(because the binding is low ☹)

↓Km $\frac{1}{\alpha}$ ↑Affinity

↑Km $\frac{1}{\alpha}$ ↓Affinity

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↑Km = ↓Affinity
↓Km = ↑Affinity

5-Michaelis-Menten

Km = Michaelis Menten Constant

- Shows affinity of an enzyme to its specific substrate
- Substrate concentration at which Vo is exactly ½ Vmax

[S]>>>Km

Is equivalent to….. Vmax, Zero order kinetics, All active sites are bound, reaching the plateau, ect…

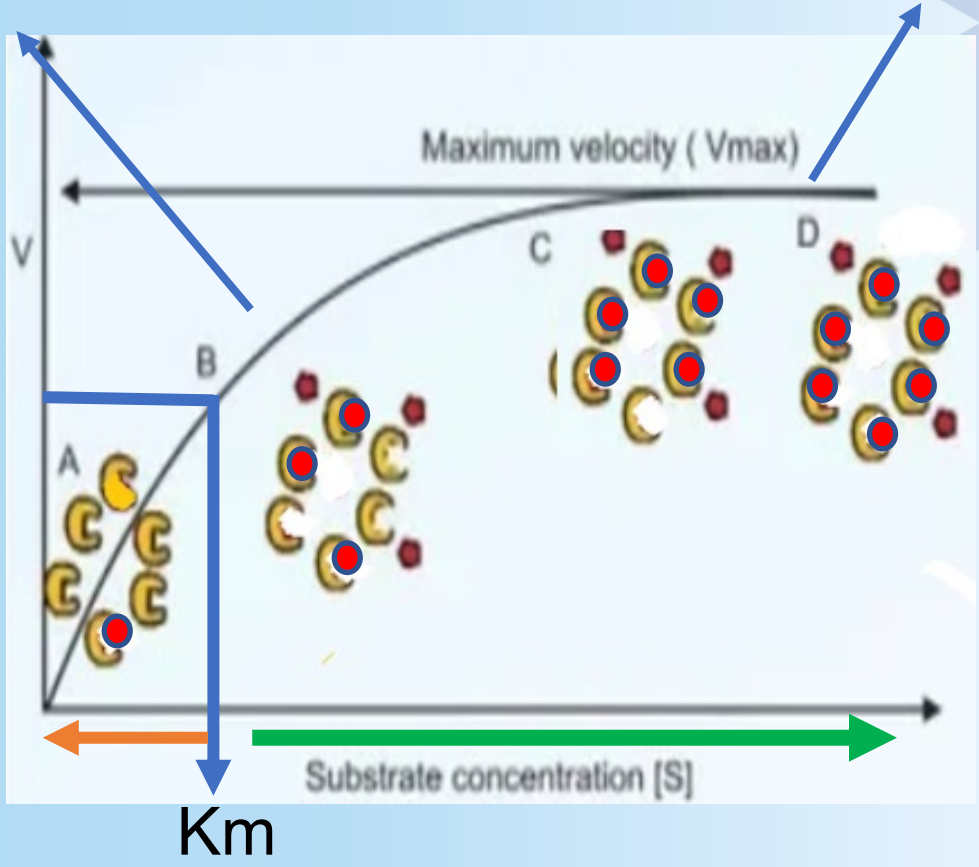
[S] = Km

[S]<<<Km

Is shows us that…. First order kinetics, the enzymes are not fully saturated, there could be a low affinity to the substrate ect…

First Order
Rate = k [A]^1

Zero Order
Rate = k[A]^0



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↑Km = ↓Affinity
↓Km = ↑Affinity

5-Michaelis-Menten

What is the initial velocity for the Michaelis Menten equation with the following values: Vmax = 4 mM/min, Km = 1mM, S = 3mM

$$V_o = \frac{V_{max}[S]}{K_m + [S]}$$

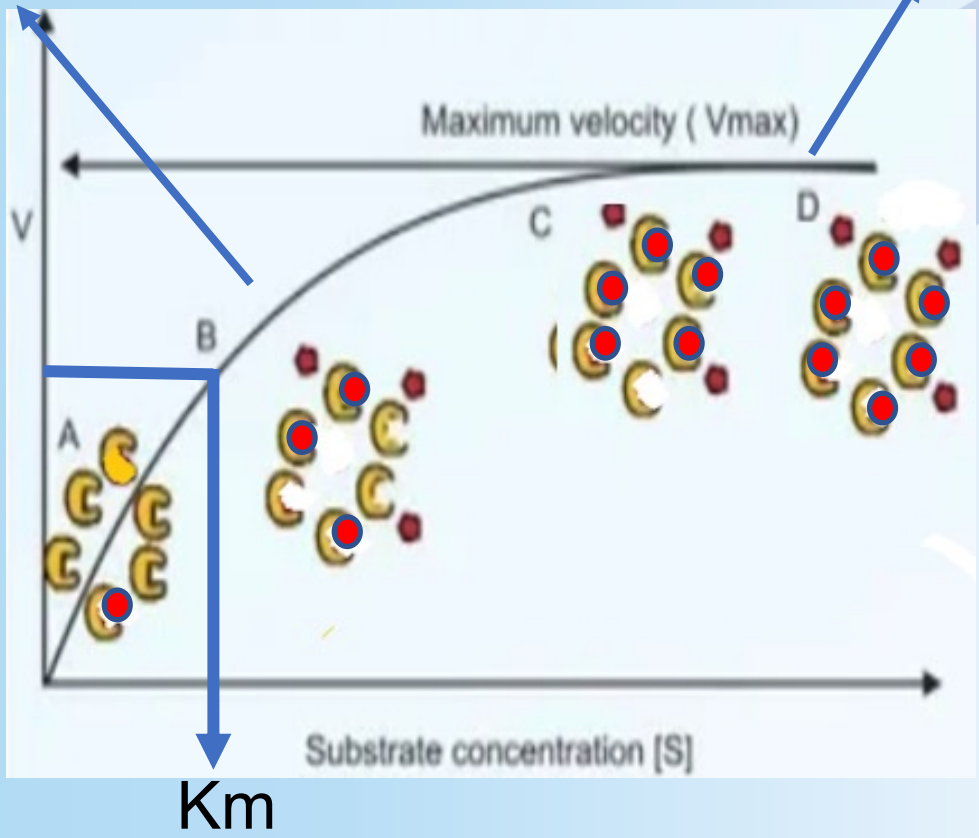
$$Vo = \frac{4 \times [3]}{1 + [3]}$$

$$Vo = \frac{12}{4}$$

Vo = 3 mM

First Order
Rate = k [A]^1

Zero Order
Rate = k[A]^0



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↑Km = ↓Affinity
↓Km = ↑Affinity

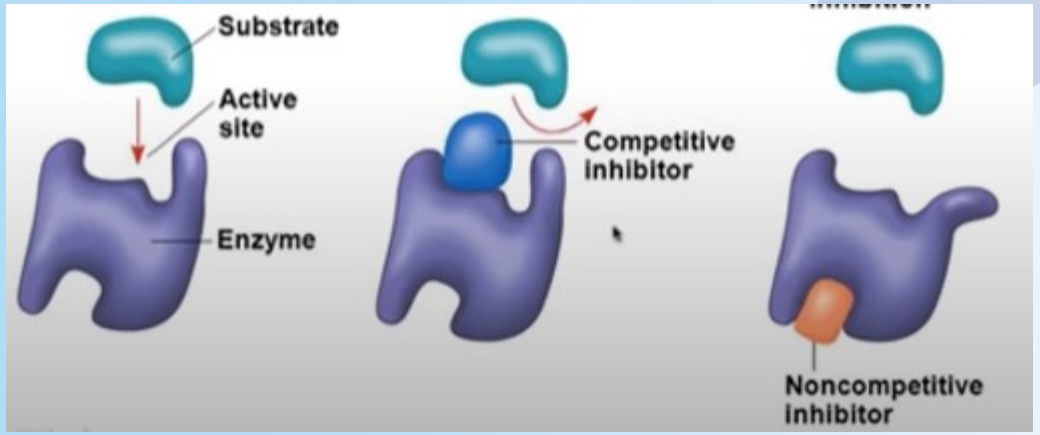
$$V_o = \frac{V_{max}[S]}{K_m + [S]}$$

6-Inhibition of Enzyme Activity

Competitive & Noncompetitive Inhibition

2x Major Groups:

-**Reversible Inhibitors** =
Bind to Enzyme via non-covalent bonds
6a. Competitive Inhibition
6b. Noncompetitive Inhibition



-Irreversible **Inhibitors** = Bind to Enzyme via covalent bonds
Suicide Inhibition

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↑Km = ↓Affinity
↓Km = ↑Affinity

$$V_o = \frac{V_{max}[S]}{K_m + [S]}$$

6-Inhibition of Enzyme Activity
Competitive & Noncompetitive Inhibition

2x Major Groups:

-Reversible Inhibitors

-Irreversible Inhibitors = Bind to Enzyme via covalent bonds

Suicide Inhibition

Examples:

Aspirin binds to COX-1 & COX-2

Inhibits PGs & TXA2 synthesis ➔ Reduces Inflammation, and antiplatelet function

Sarin (nerve gas) binds to AChEsterase

Inhibits AChE at Neuromuscular junction ➔ Buildup of Ach ➔ Cholinergic crisis

Penicillin binds to active site of Glycopeptide transpeptidase (bacteria)

Inhibition of crosslinkage formation reaction between peptidoglycan molecules

↑Km = ↓Affinity
↓Km = ↑Affinity

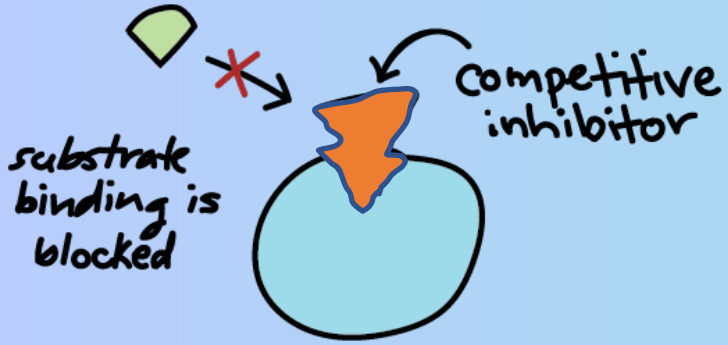
$$V_o = \frac{V_{max}[S]}{K_m + [S]}$$

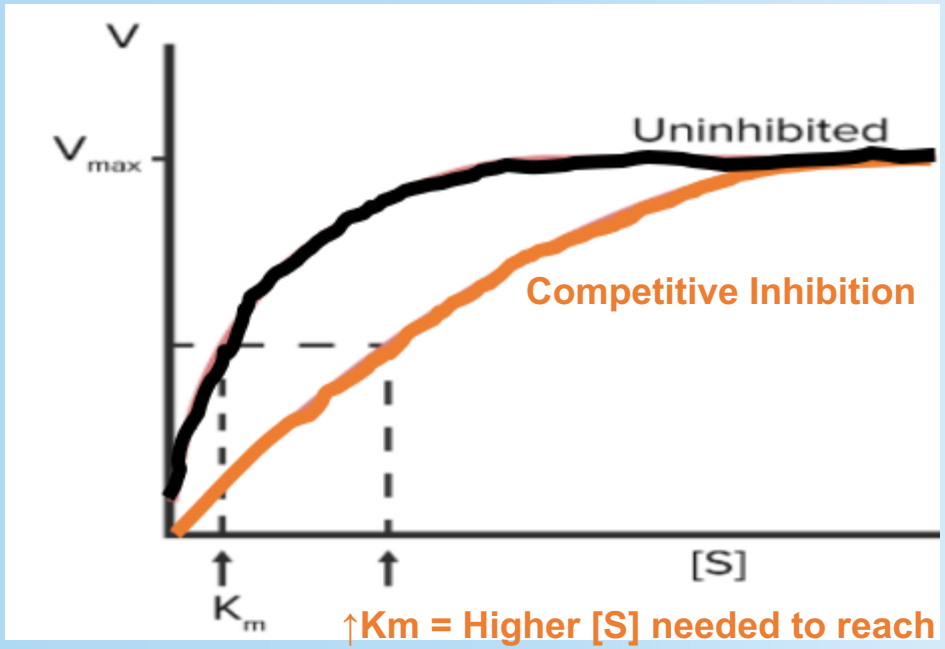
6-Inhibition of Enzyme Activity

Competitive & Noncompetitive Inhibition (Competition to active site)

6a. Competitive Inhibition

-Competes for the active site = ↑Km
-Inhibition is overcome by ↑ [S]= Normal Vmax!





↑Km = Higher [S] needed to reach ½ Vmax

↑Km =
Killer moustache

-↑ Km
-Vmax = Normal

ONLY NEED TO KNOW
What happens to either
Km or Vmax!

1.Ex: Statins = Which Inhibits HMG-CoA Reductase
Resulting in ↓Cholesterol
2. Ex: -PRIL (Captopril) = Which inhibits AngiotensinConvertingEnzyme
leading to a failure to convert Angiotensin 1→Angiotensin 2
Leading to vasodilation.

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↑Km = ↓Affinity
↓Km = ↑Affinity

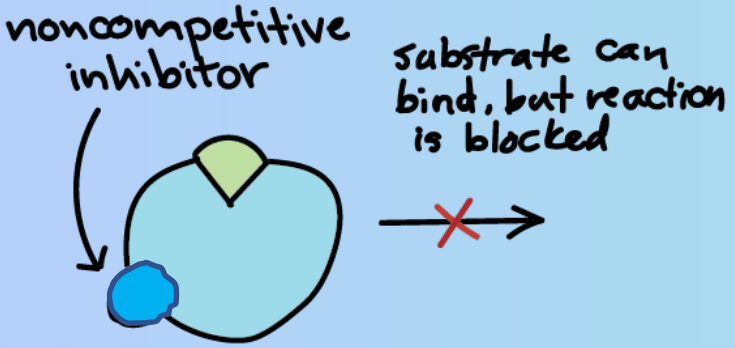
$$V_o = \frac{V_{max}[S]}{K_m + [S]}$$

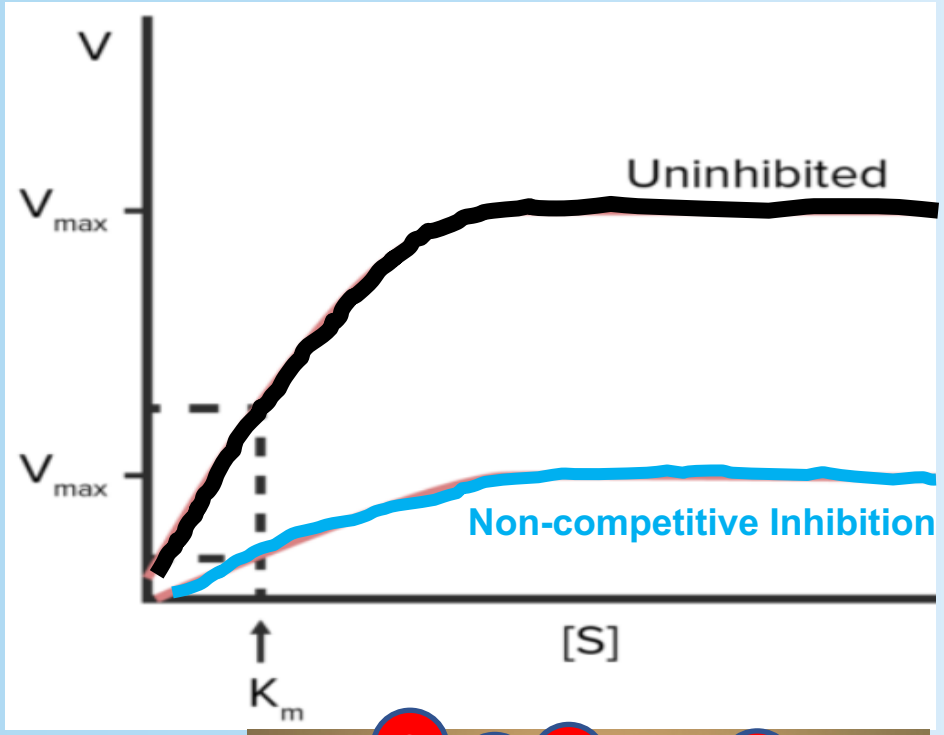
6-Inhibition of Enzyme Activity

Competitive & Noncompetitive Inhibition (Think active site!!!)

6b. Non-competitive Inhibition

-Binds to DIFFERENT site on the enzyme
-Inhibition can't be overcome by ↑ [S]
-Don't interfere with the binding of [E] to [S] = Km Normal
-↓ the reaction to proceed as efficiently=↓Vmax
-Can bind on either a free enzyme OR ES!





-↓ Vmax
-Km is Normal

ONLY NEED TO KNOW
What happens to either Km or Vmax!

↑Km = ↓Affinity
↓Km = ↑Affinity

$$V_o = \frac{V_{max}[S]}{K_m + [S]}$$

Contents!

↑Km = ↓Affinity
↓Km = ↑Affinity

7. Lineweaver Burk Plot

$$V_o = \frac{V_{max}[S]}{K_m + [S]}$$

Micheals Menten Equation
-Log graph (Hyperbolic)
-Gives an inaccurate Vmax

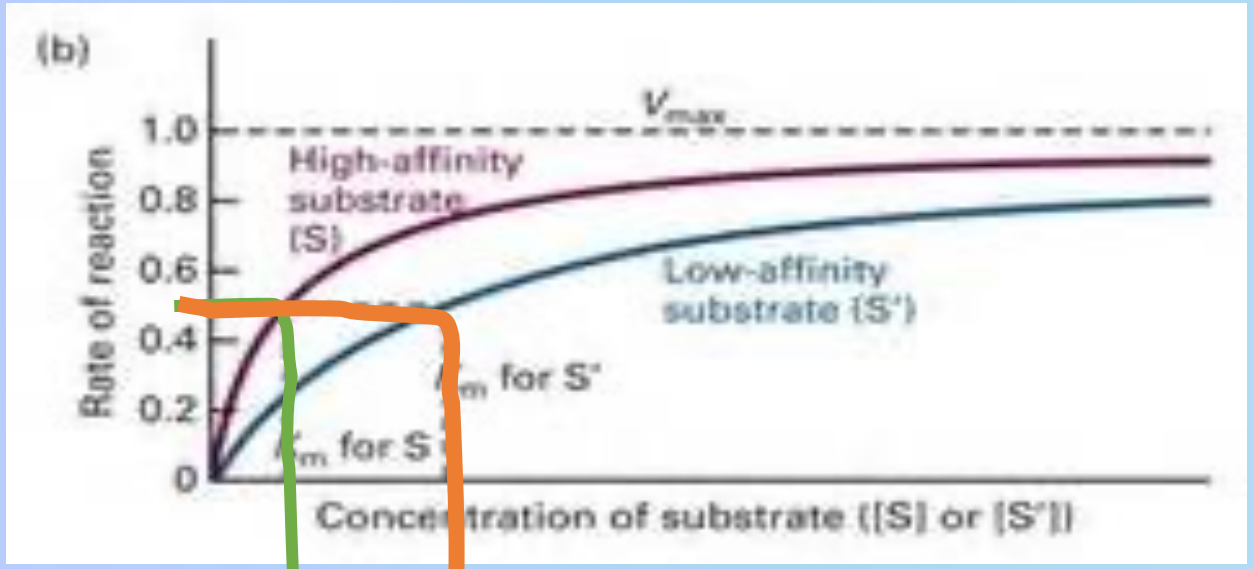
$$V_o = \frac{V_{max}[S]}{K_m + [S]}$$

Reciprocal

Lineweaver Burk Plot
-Linear graph
-Accurate Vmax

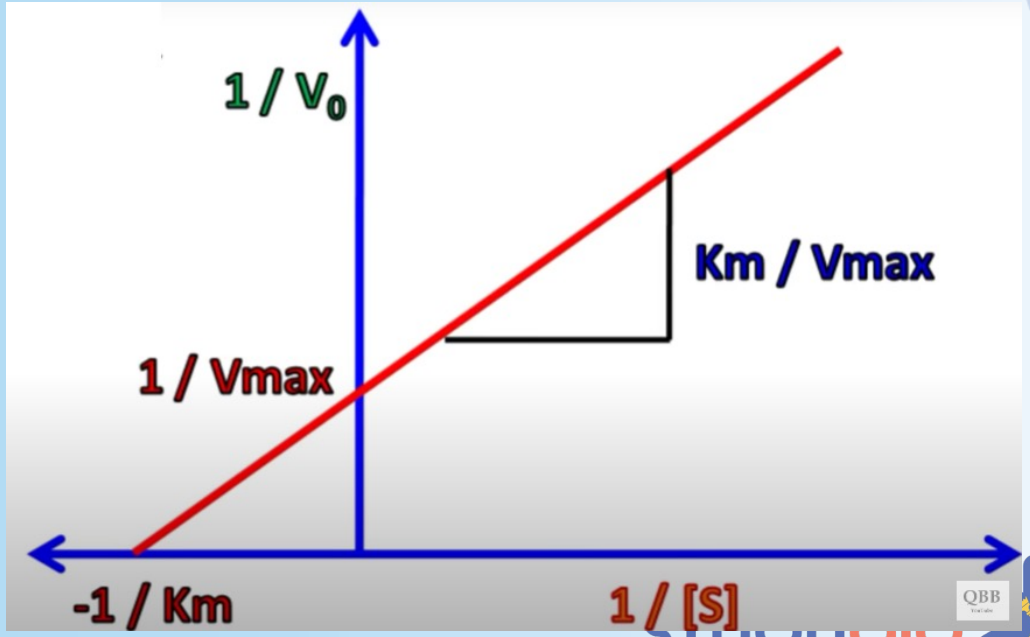
$$\frac{1}{V_0} = \frac{K_m}{V_{max}[S]} + \frac{1}{V_{max}}$$

$$Y = m\,x + c$$



↓Km $\frac{1}{\alpha}$ ↑Affinity

↑Km $\frac{1}{\alpha}$ ↓Affinity



↑Km = ↓Affinity
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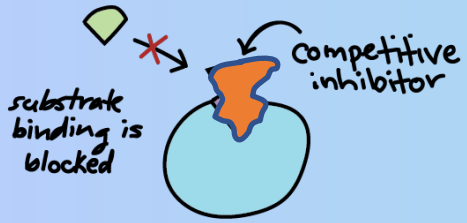
7. Lineweaver Burk Plot

$$V_0 = \frac{V_{max}[S]}{K_m + [S]}$$

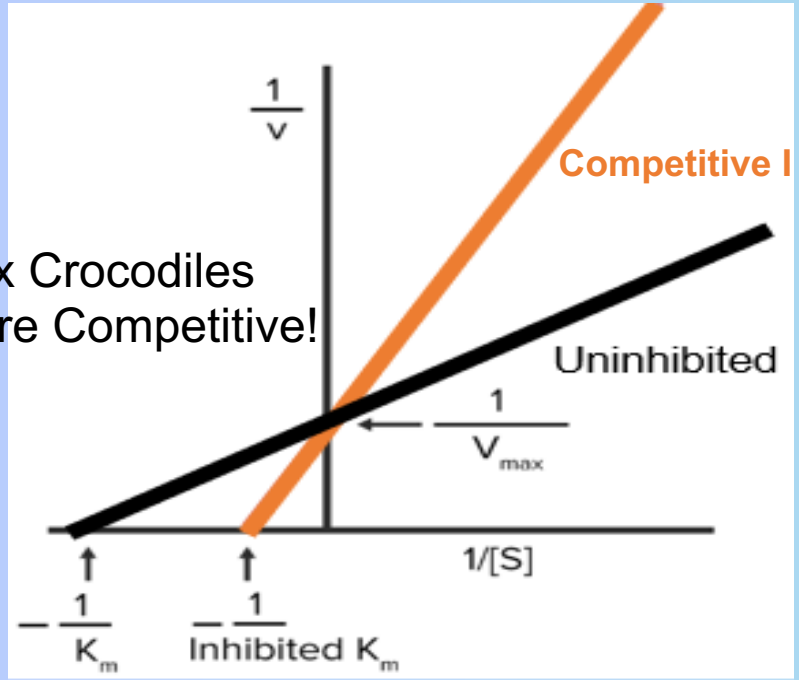
Lineweaver-Burk Equation $\frac{1}{v_0} = (\frac{K_m}{V_{max}})\frac{1}{[S]} + \frac{1}{V_{max}}$



Competitive Inhibitor

-Km↑ (Normal Vmax)

-Ex: Statin([-]HMG-CoA Reductase),
-Pril([-]AngiotConvertEnzyme)

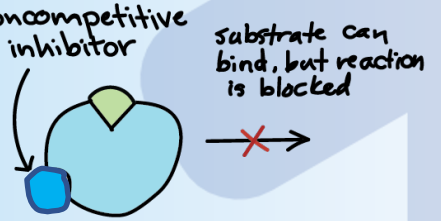


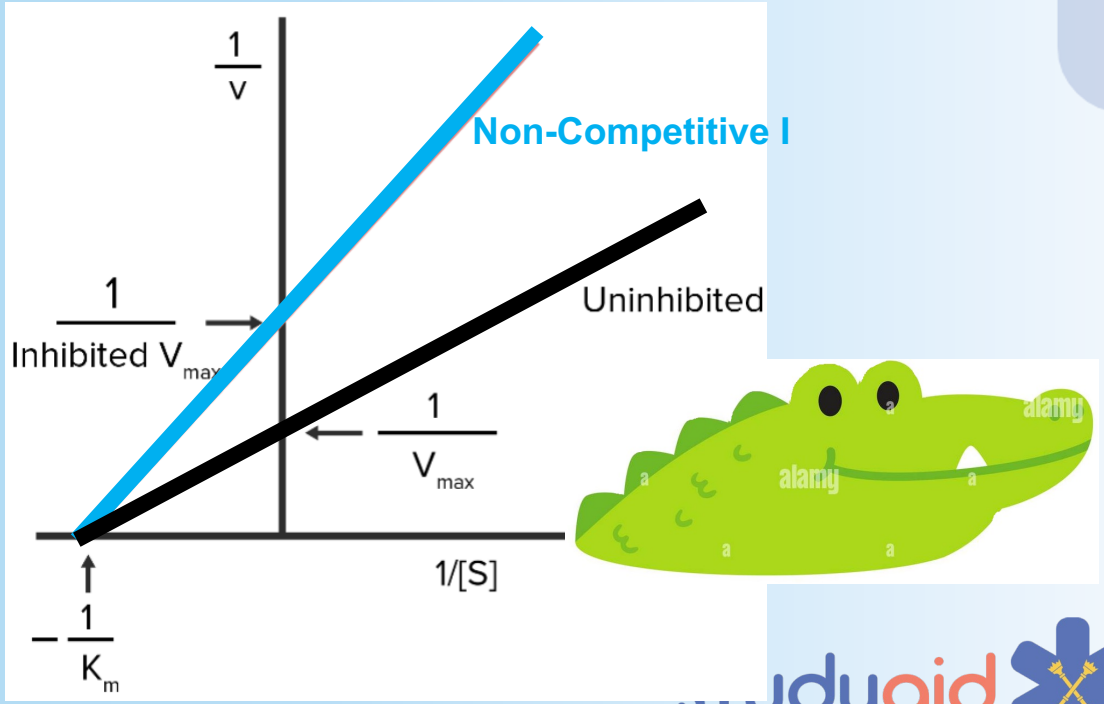
2x Crocodiles
Are Competitive!

Non-Competitive Inhibitor

-Vmax↓ (Normal Km)

-Ex:





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↑Km = ↓Affinity
↓Km = ↑Affinity

7. Lineweaver Burk Plot

$$V_o = \frac{V_{max}[S]}{K_m + [S]}$$

What is the Km In line A?

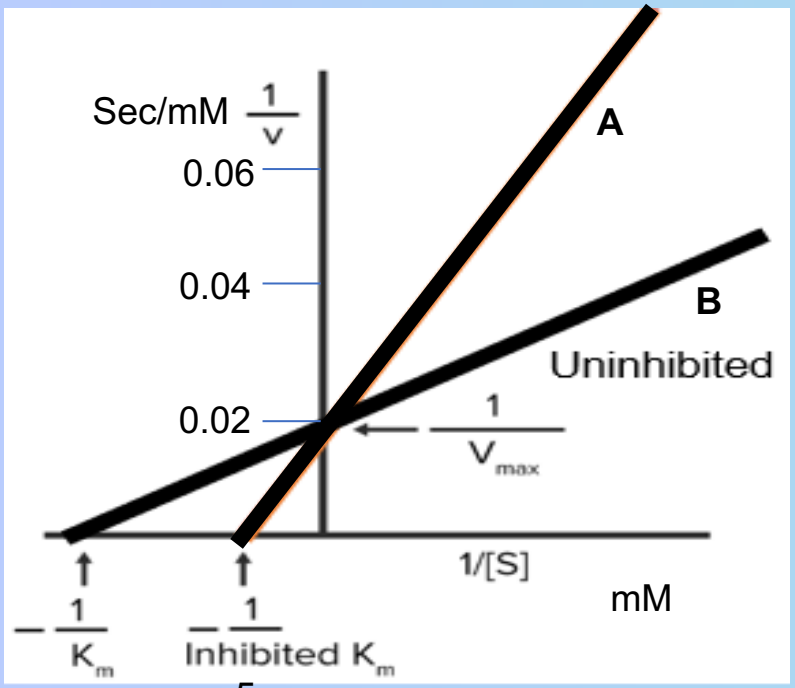
-1/Km = -5
1/Km = 5
Km = 1/5
Km = 0.2 mM

What is the value of Vmax In line A?

1/Vmax = 1/0.02 = 50

Or

1/Vmax = 2/100 = 1/50 ➔ 50
Vmax = 50 Sec/mM



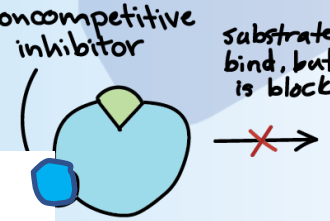
The Figure below shows plots of 1/[S] for 1/[V] according to The lineweaver Burk plot, what could this figure represent?

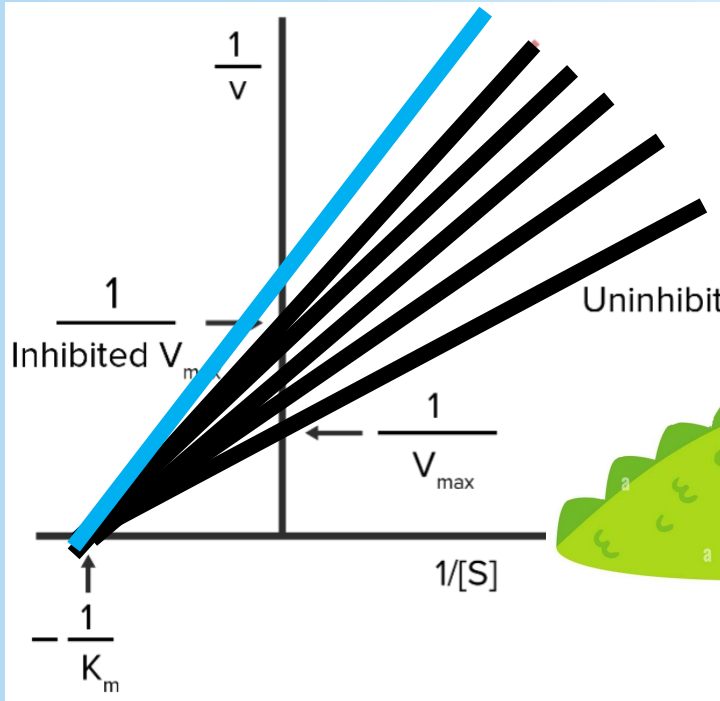
a. The inhibition of HMG-CoA reductase by statins
b. The effects of a competitive inhibitor
c. The effects of Ramipril which inhibits Angiotensin converting Enzyme
d. The effects of a non-competitive inhibitor

Non-Competitive Inhibitor

-Vmax↓

-Km is the same!





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↑Km = ↓Affinity
↓Km = ↑Affinity

8. Regulation of Enzyme Activation

$$V_0 = \frac{V_{max}[S]}{K_m + [S]}$$

Lineweaver-Burk Equation $$\frac{1}{v_0} = \left(\frac{K_m}{V_{max}}\right)\frac{1}{[S]} + \frac{1}{V_{max}}$$

A. **Allosteric binding** (Fast, seconds-minutes)
    i. Homotrophic effectors
    ii. Heterotrophic effectors

B. **Regulation of enzymes by covalent modification (Reversible)**
    i. Phosphorylation and Dephosphorylation
    ii. Response of enzyme to Phosphorylation

Zymogen Activation

Induction and repression of enzyme synthesis via gene expression(Slow, hours-days)

Cofactors/Coenzymes

Substrate availability

Product inhibition (feedback inhibition)

Compartmentation

↑Km = ↓Affinity
↓Km = ↑Affinity

8. Regulation of Enzyme Activation

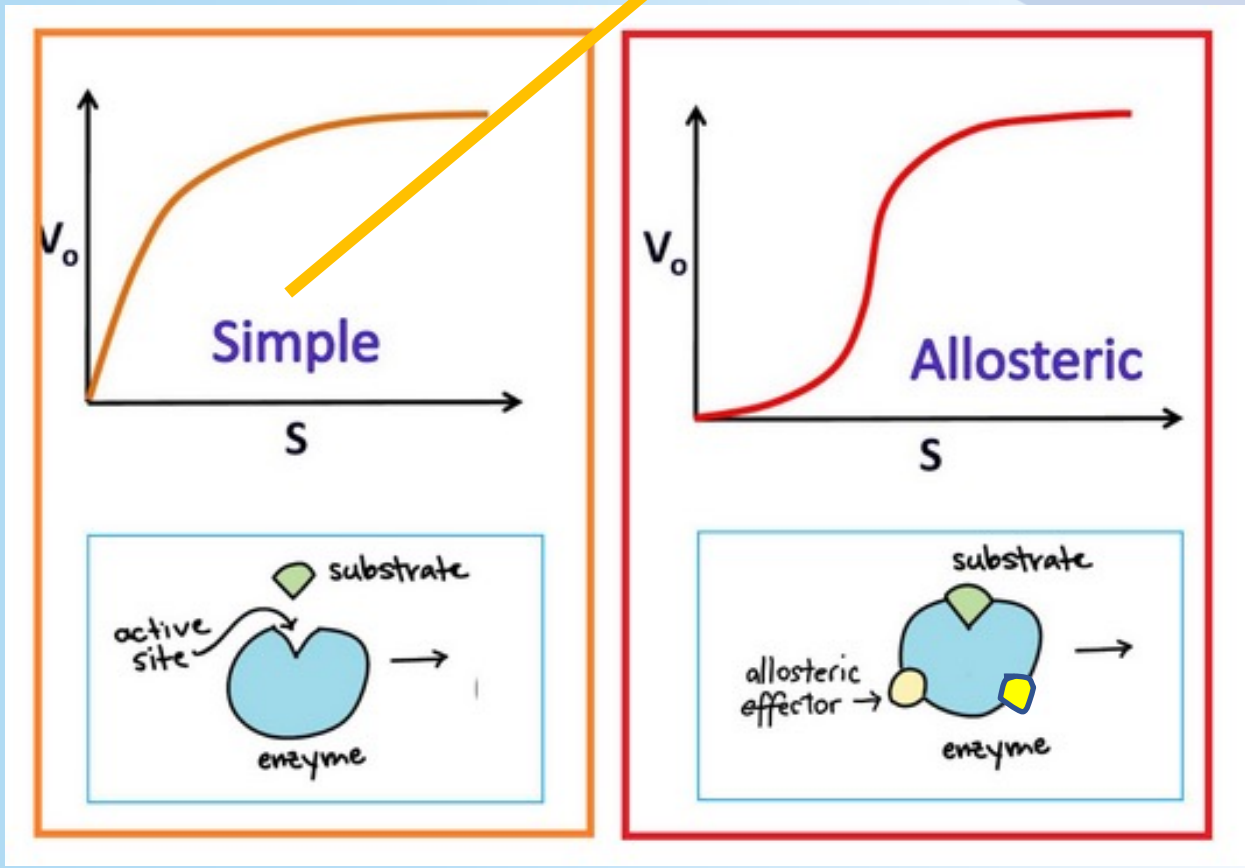
$$V_o = \frac{V_{max}[S]}{K_m + [S]}$$

Lineweaver-Burk Equation $\frac{1}{v_0} = (\frac{K_m}{V_{max}})\frac{1}{[S]} + \frac{1}{V_{max}}$

8A. Allosteric binding (Fast, seconds-minutes)
Create Sigmoidal curves.

-**Allosteric Enzyme** =
A multi-subunit protein whose activity is affected by binding of other molecules (effectors)

-**Allosteric Effectors** =
Molecules that bind to allosteric enzymes and alter the **function/conformation/binding affinity**.
Can be either activators or inhibitors.



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↑Km = ↓Affinity
↓Km = ↑Affinity

8. Regulation of Enzyme Activation

$$V_0 = \frac{V_{max}[S]}{K_m + [S]}$$

Lineweaver-Burk Equation $$\frac{1}{v_0} = \left(\frac{K_m}{V_{max}}\right)\frac{1}{[S]} + \frac{1}{V_{max}}$$

8A. Allosteric binding (Fast, seconds-minutes)

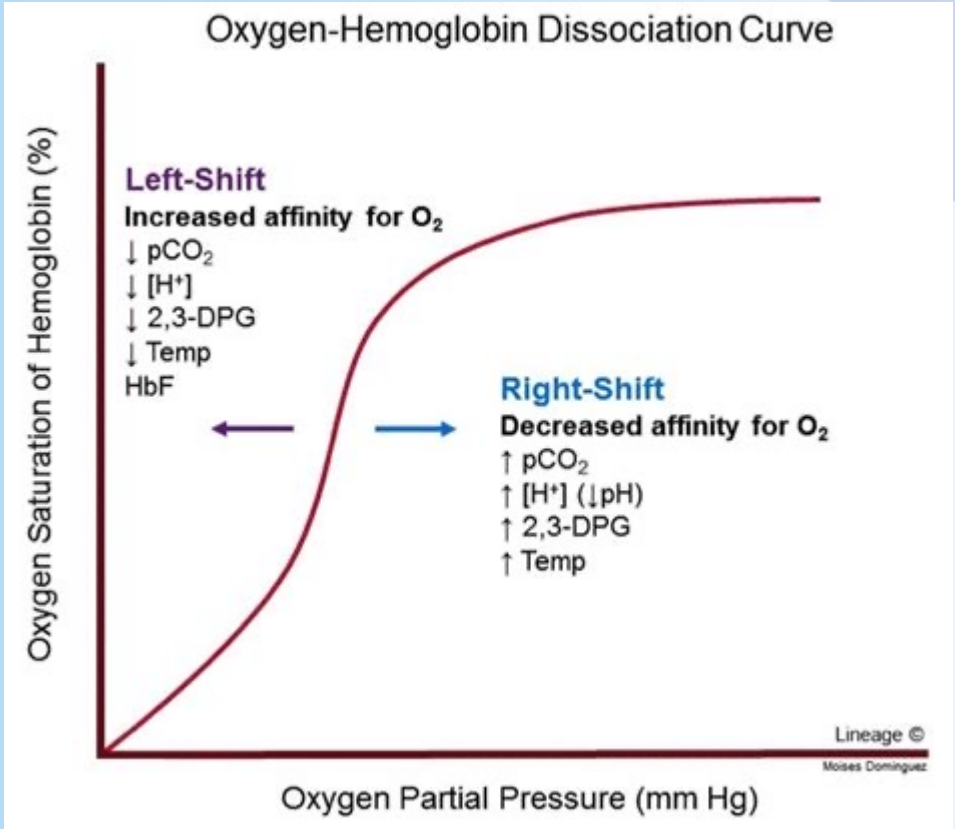
-Allosteric Enzyme
-Allosteric Effectors (x2)

i. Homotrophic Effectors

-Binding of the substrate (primary ligand) at 1 site affects the binding of THAT SAME LIGAND at another site
-ex: Hgb & O2 ➔ Cooperativity (Binding of 02 at 1 site will ↑Affinity at other sites)

ii. Heterotrophic Effectors

-Binding of a ligand DIFFERENT from the primary ligand has an affect on the enzymes' affinity for the primary ligand
-ex: ↑ H, CO2, 2,3BPG (Bohr Effect)[Not primary ligand]
In this example will ↓Affinity for O2
Shift to the RIGHT



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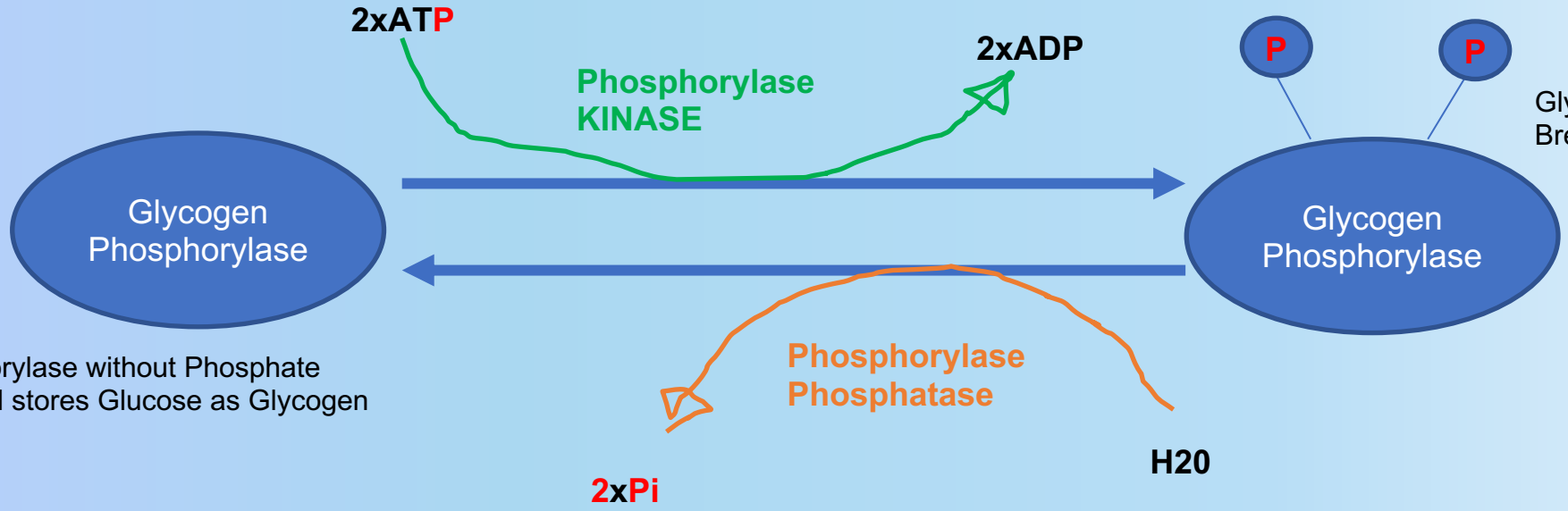
8B. Regulation of enzymes by covalent modification (Reversible)
-Phosphorylation doesn't always activate an enzyme/protein!!!
-Depends on which enzyme/protein

Phosphorylation
-Adds Phosphate group to an enzyme
-Phosphorylase Kinase

Dephosphorylation
-Removing Phosphate group from an enzyme
-Phosphorylase Phosphatase



Glycogen Phosphorylase Is Active
Breaks down Glycogen into Glucose

Glycogen Phosphorylase without Phosphate
Is now inactive and stores Glucose as Glycogen

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↑Km = ↓Affinity
↓Km = ↑Affinity

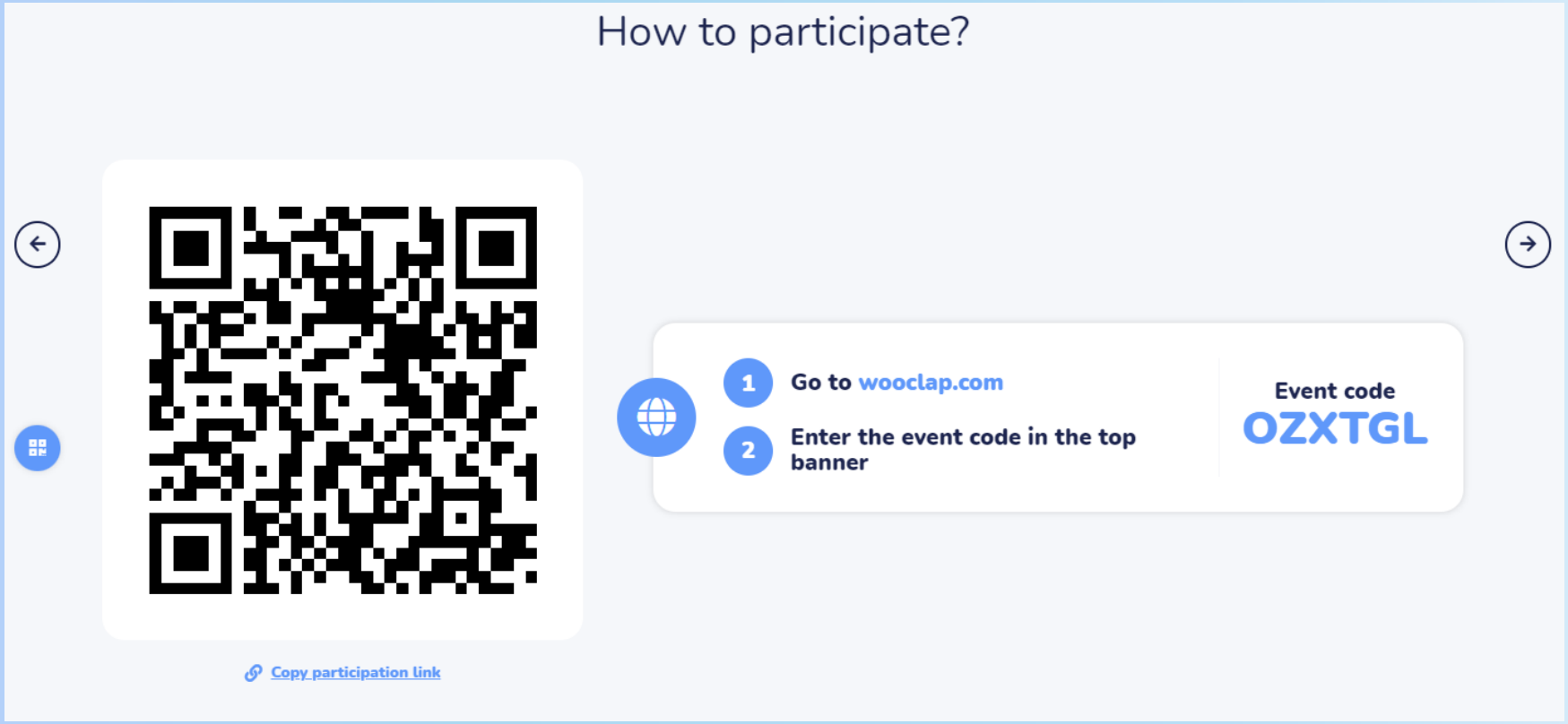
$$V_0 = \frac{V_{max}[S]}{K_m + [S]}$$

Lineweaver-Burk Equation $\frac{1}{v_0} = (\frac{K_m}{V_{max}})\frac{1}{[S]} + \frac{1}{V_{max}}$

Contents!

Questions

https://www.wooclap.com/OZXTGL



↑Km = ↓Affinity
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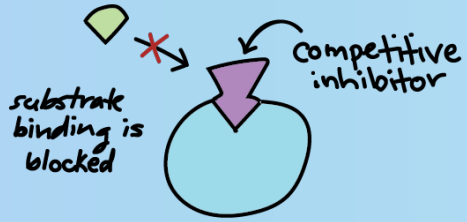
$$V_0 = \frac{V_{max}[S]}{K_m + [S]}$$

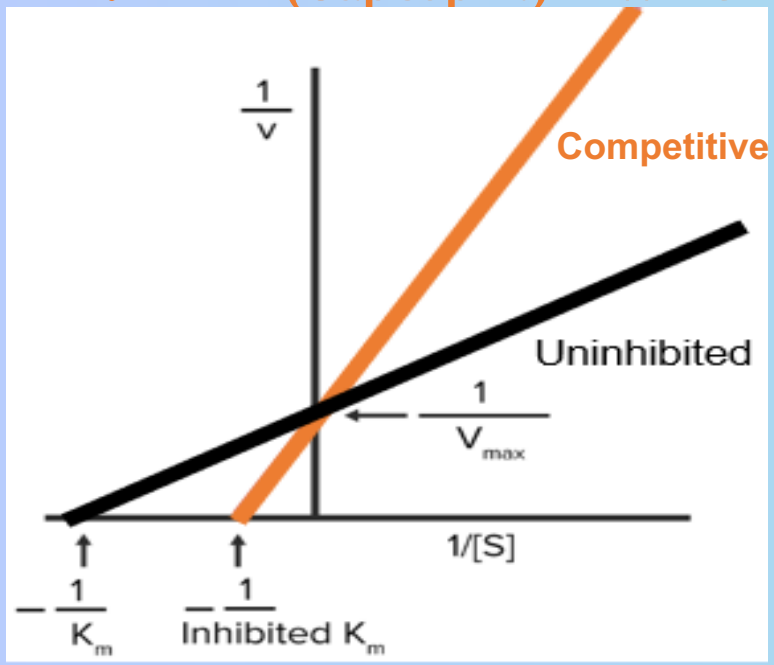
Good luck!

Lineweaver-Burk Equation $\frac{1}{v_0} = (\frac{K_m}{V_{max}})\frac{1}{[S]} + \frac{1}{V_{max}}$

Competitive I



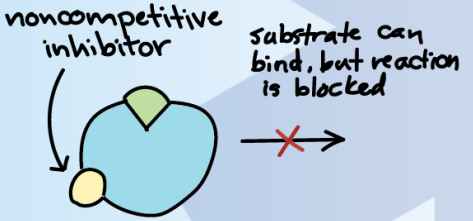
-Km↑
-Vmax=Normal
-Ex: Statins = (-) HMG-CoA Reductase
- Ex: -PRIL (Captopril) = Which inhibit ACE

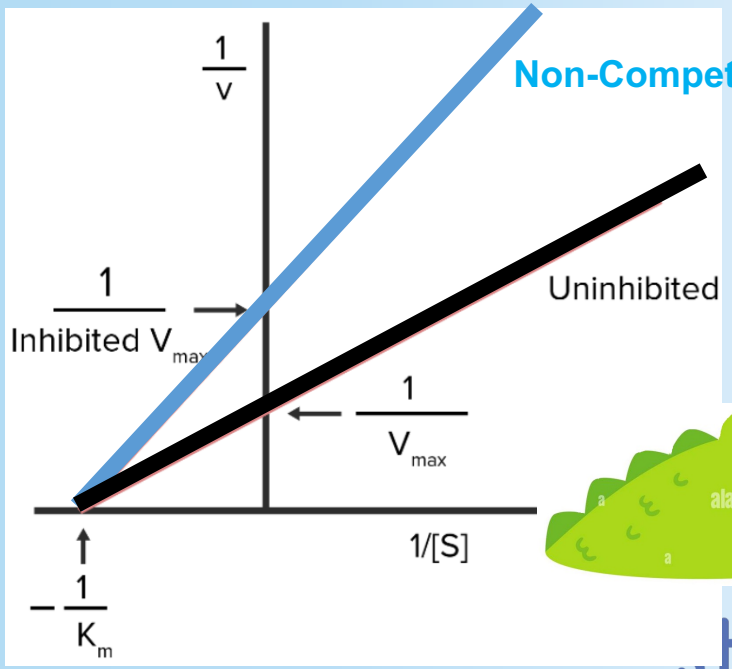


(Line always goes up↑)

Non-Competitive I

-Vmax↓
-Km=Normal





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