

# CH40001 Biochemical Engineering

## Chapter 3. Enzyme Kinetics

Saikat Chakraborty  
Department of Chemical Engineering,  
Indian Institute of Technology

# Epistemic Qs.

- 
1. How do enzymes/catalysts act, thermodynamically?
  2. How does their thermodynamic role accelerate reaction kinetics?
  3. How does enzymes/catalyst act stereochemically?
  4. What's the difference between reversible and irreversible reactions?
  5. Why does pH and temperature affect enzyme activity and therefore reaction kinetics?
  6. Do enzymes/catalysts take part in the reaction?
  7. What's an enzymes relationship with it's substrates?

# Epistemic Qs.

8. What's the role of evolutionary biology in determining the structure of lignocelluloses in plant cell walls?
9. How do enzymes connect to evolutionary biology?
10. What kind of reactors can attain steady state?
11. Why lumped kinetics is more advantageous than detailed kinetics?
12. What are the uses of asymptotic solutions to equations?
13. When is Quasi-steady state (QSS) assumption valid for a species (or several species) participating in a reaction network?
14. Reaction networks: series/parallel/series-parallel?

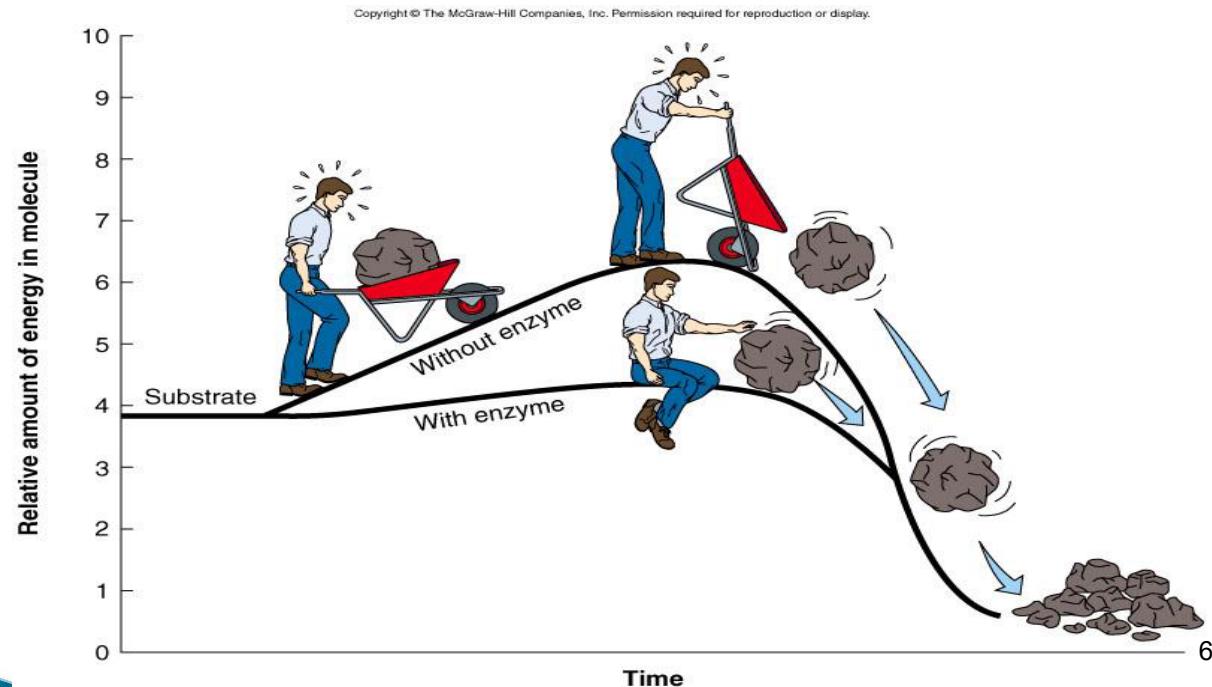
# Epistemic Qs.

15. How does mixing limitation influence reaction rate (kinetics)?
16. How to manipulate reactor mixing/type to regulate product distribution?
17. What is the most potent inhibition in Biochemical systems? Why?
18. How to calculate the maximum substrate loading in Biochemical reactions?
19. How to calculate the optimum pH for an enzymatic reaction?
20. What determines the rate of transport of enzymes to solid substrate surface in a well-mixed reactor?

**Epistemic Qs 1.**  
**How do enzymes/catalysts act, thermodynamically?**

# Energy

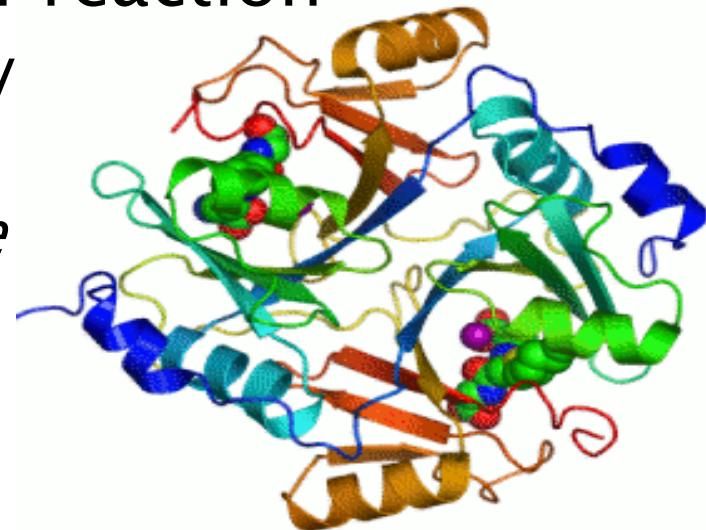
- ▶ All living things require energy.
  - *Nutrients* are one source of energy, as well as being molecules organisms require to grow, reproduce or repair
- ▶ *Biochemical reactions* are the processes used for the formation, breakdown and rearrangement of molecules to provide organisms with energy



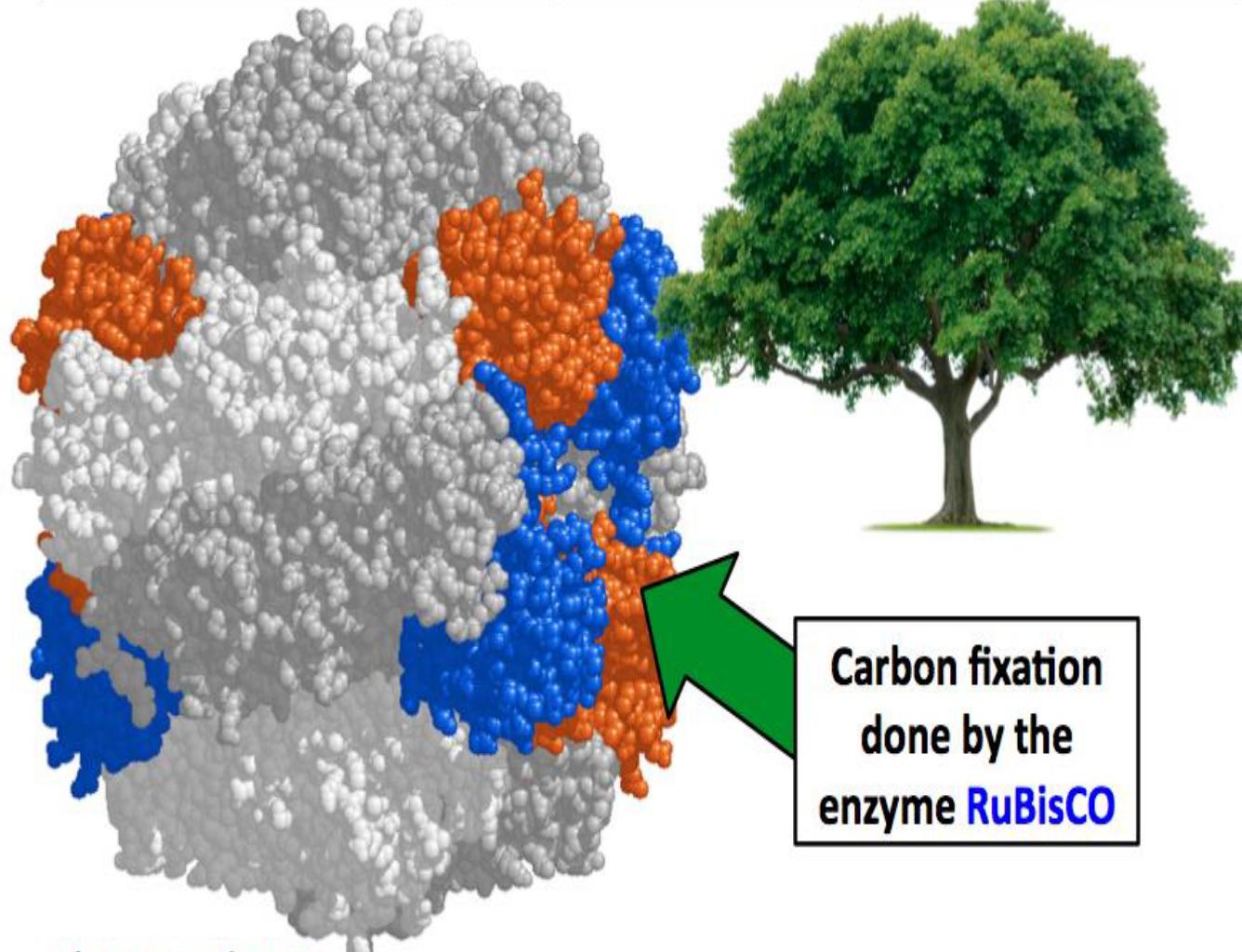
**Epistemic Qs 2.**  
**How does their thermodynamic role accelerate  
reaction kinetics?**

# Enzymes are biological catalysts

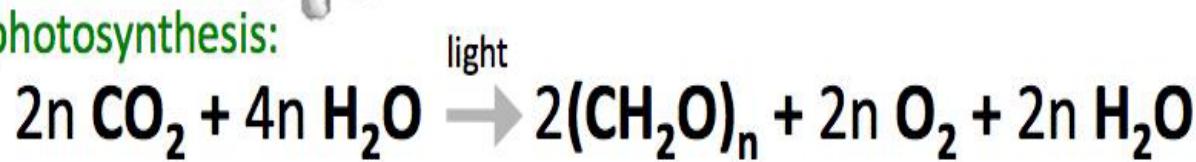
- ▶ A *catalyst* is a chemical that speeds up the reaction but is not used up in the reaction
  - Lowers the activation energy needed to start a reaction
  - Is not used up during the reaction
  - Is unchanged after a reaction
- ▶ *Enzymes* act as catalysts. Enzymes are proteins that speed up a rate of reaction
  - Found in cells throughout the body
  - Lowers activation energy
  - Names of enzymes will end in *-ase*
  - They are specific.



## Ribulose-1,5-bisphosphate carboxylase oxygenase



photosynthesis:



# Enzymes

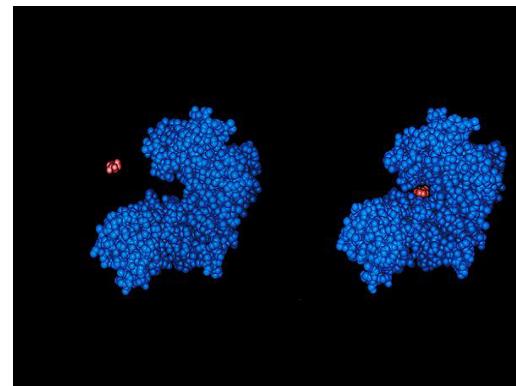
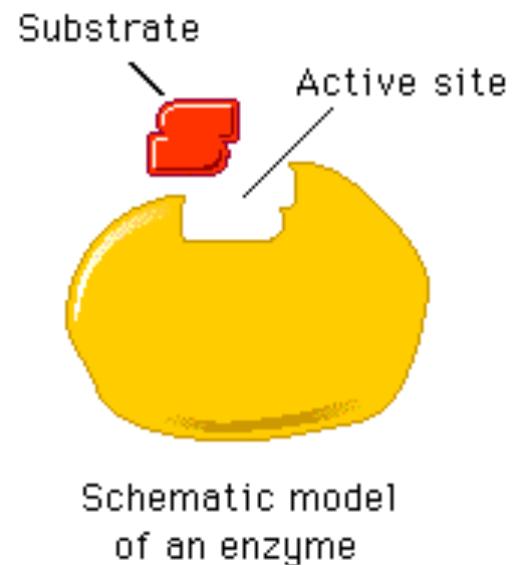
- They are proteins, RNA or DNA that catalyze biological reactions.
- It increases the rate of biological reactions  $10^4$  to  $10^{12}$  times the rate in the absence of the catalyst (many important biochemical reactions do not occur appreciably in the absence of an enzyme).
- Specificity: Enzymes are specific to reactant molecules known as substrates which interact at a specific site on the enzyme, often with high affinity. The high affinity finding allows enzymes to function effectively in a solution containing a large no. of biological molecules at low concentration.
- The activity of enzymes can be regulated in several ways, offering control over the rate and amount of product formed . Examples of regulation include
  - (a) cofactor , which binds to the enzyme.
  - (b) using a reaction product that inhibits the reaction.

**Epistemic Qs 6.**

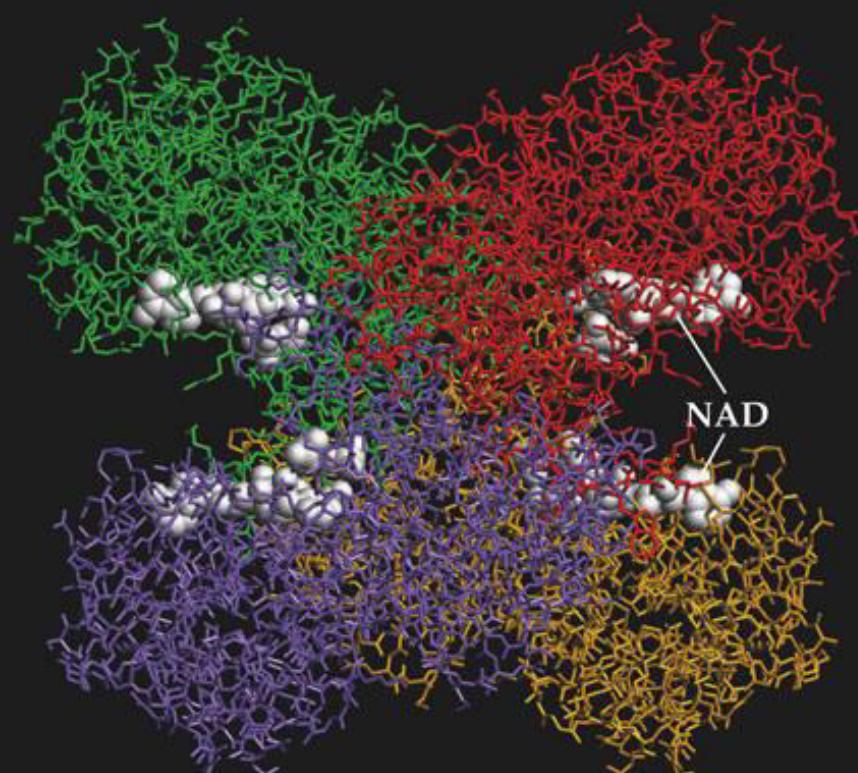
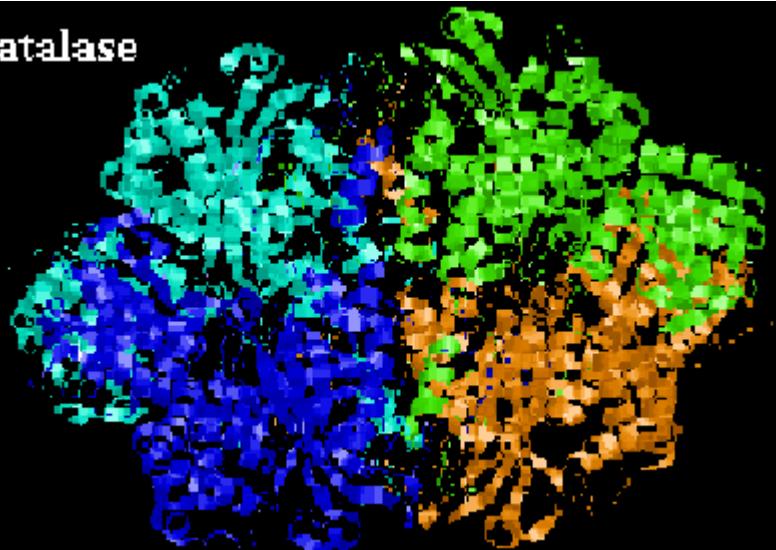
**Do enzymes/catalysts take part in the reaction?**

# How Enzymes work : Biochemistry of Enzyme Function

- Enzymes catalyze biochemical reactions following the binding of one or more substrates to the active site of the enzyme. An active site interacts specifically with the substrate, provides appropriate orientation of the reacting molecules and alters the local electro-dynamic environment to make the occurrence of the reaction more favorable.
- Specific amino-acid side chains serve as catalytic agents facilitating bond breakage or formation of the product.
- Molecular biology techniques (such as site-directed mutagenesis) are used to determine the specific structure-function relationship).

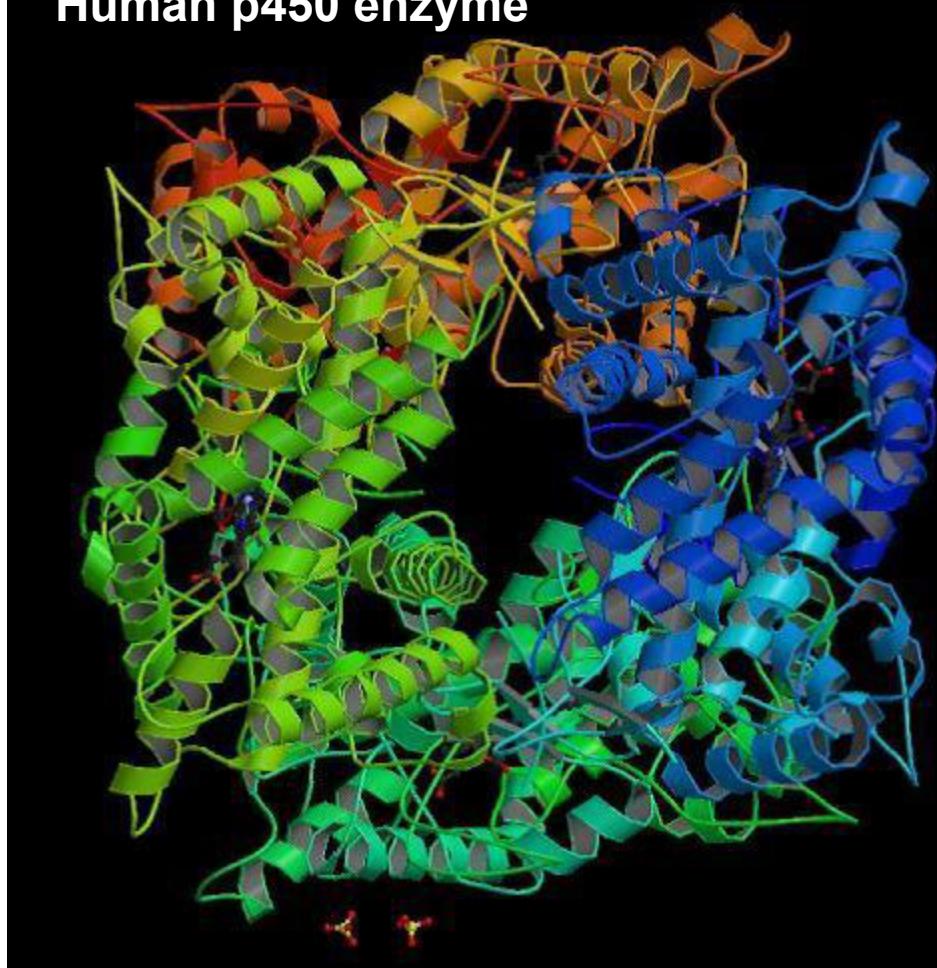


Catalase



Glyceraldehyde-3-phosphate dehydrogenase

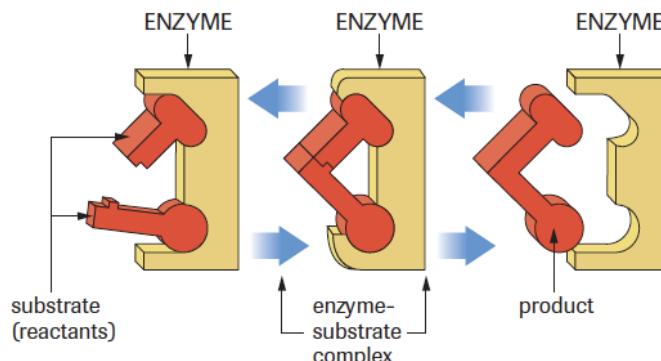
Human p450 enzyme



## Enzyme Structures

# CATALYSTS AND REACTION RATE

- How do catalysts work??
- Scientists do not really understand the actual mechanism. Catalysts are also usually discovered through trial and error.
- What they do know is that they provide an alternative, lower energy pathway from reactants to products.
- Most of the catalysts (**enzymes**) for biological reactions work by shape and orientation. They fit substrate proteins into locations on the enzyme as a key fits into a lock, enabling only specific molecules to link or detach on the enzyme.
- Almost all enzymes catalyze only one specific reaction

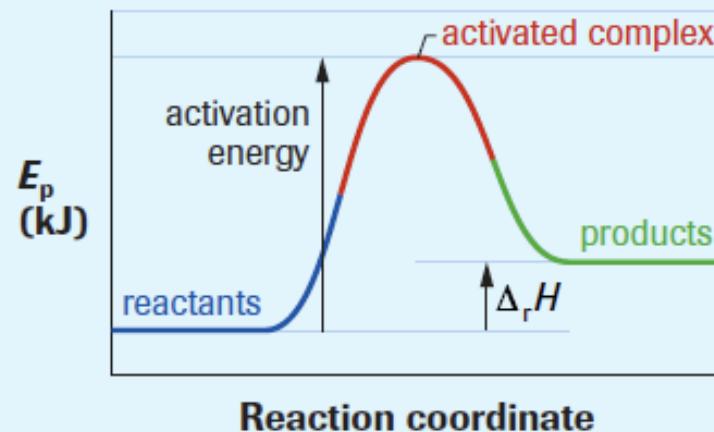


# LET'S SEE IF YOU GET IT

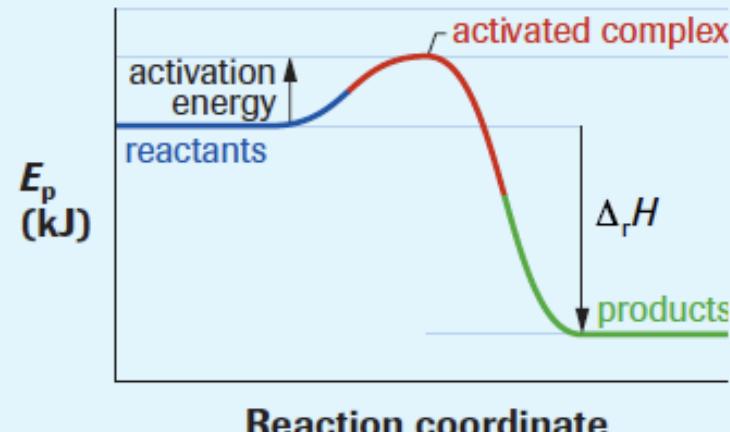
Draw energy pathway diagrams for general endothermic and a general exothermic reaction. Label the reactants, products, enthalpy change, activation energy, and activated complex.

## *Solution*

### Potential Energy Changes During an Endothermic Reaction



### Potential Energy Changes During an Exothermic Reaction



**Epistemic Qs 3.**

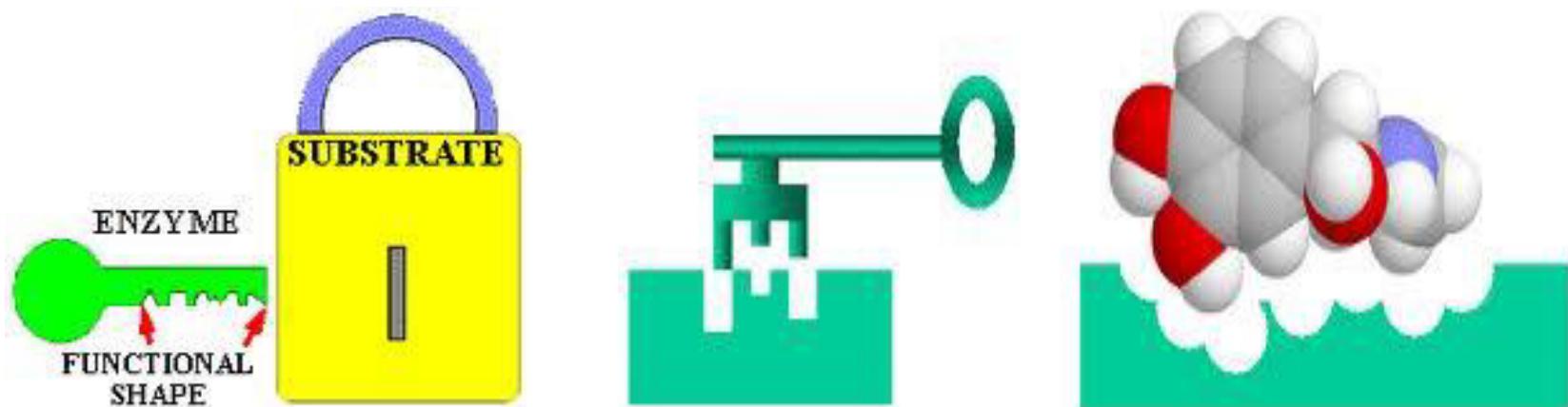
**How does enzymes/catalyst act stereo-chemically?**

**Epistemic Qs 7.**

**What's an enzymes relationship with it's substrates?**

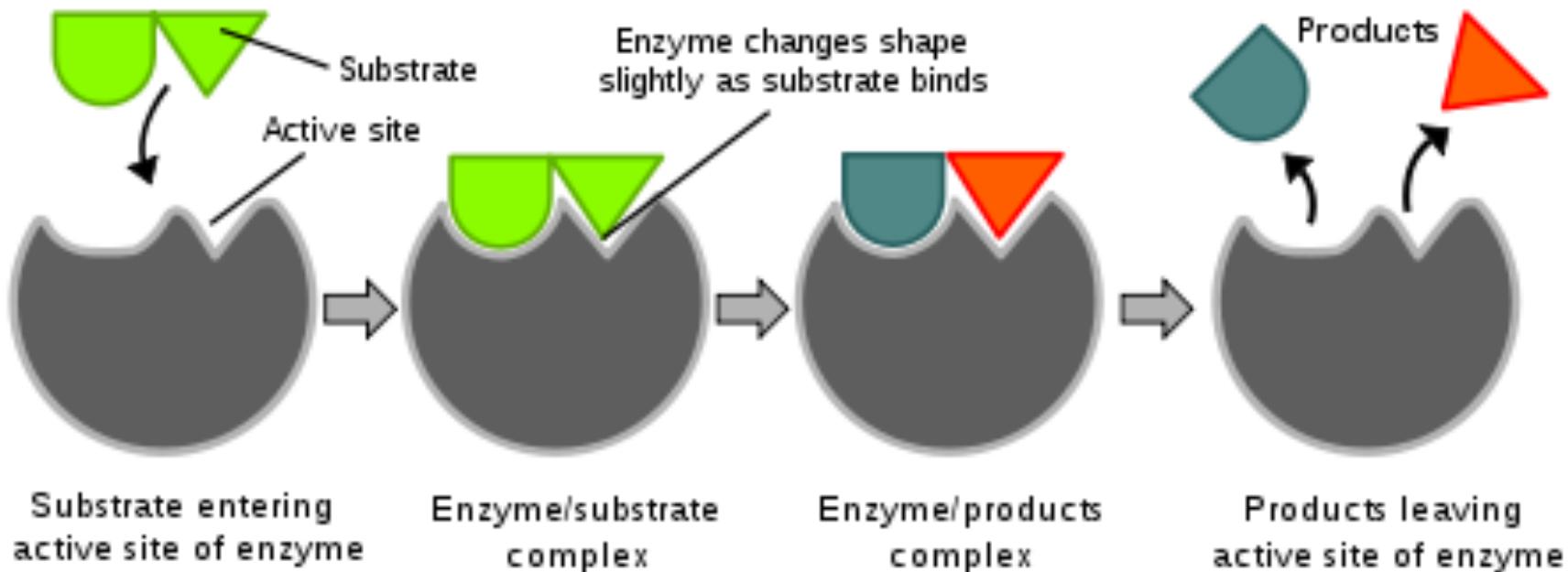
# Enzyme Form and Function

- **Lock & key Model:** The shape of an enzyme allows it to do a specific job much like a lock and key.



# Induced Fit Model

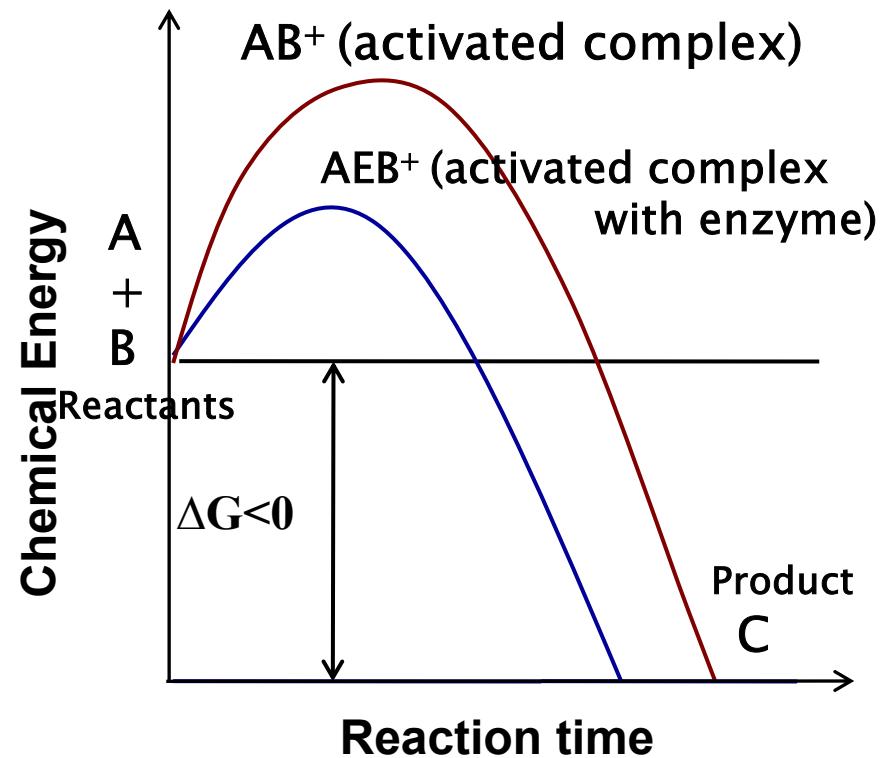
- Enzymes can form to the shape of its substrate.



[http://en.wikipedia.org/wiki/File:Induced\\_fit\\_diagram.svg](http://en.wikipedia.org/wiki/File:Induced_fit_diagram.svg)

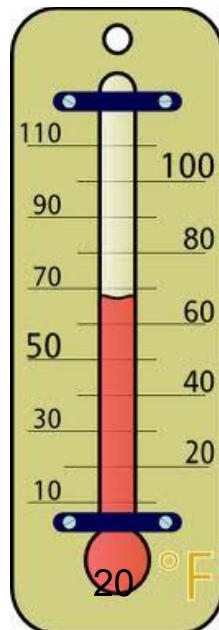
# How enzymes work: the Thermodynamics of Enzyme Activity

- For any reaction to be thermodynamically favorable ,  $\Delta G<0$ .
- However even for reactions with  $\Delta G<0$ , they are often limited by the energy barrier needed to form an activated state. As a result these reactions do not occur without *heat or catalyst*.
- Enzymes provide an alternate reaction pathway that produces an activated state of the reactants with lower energy barrier. As a result , the rate of reaction is increased significantly but the overall change in energy between the reactants and the products is not altered.



# Denaturing Enzymes

- When an enzyme is denatured it is damaged.
- Denaturing changes the shape.
- Without the correct shape enzymes won't function properly.
- HOW are enzymes denatured?
  - Temperature
  - pH



# What other factors control the action of enzymes?

- Temperature
- Water Content
- pH
- Chemicals
- Alteration of Substrates
- Alteration of Products



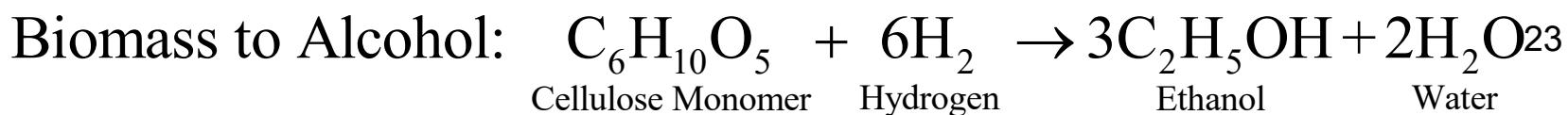
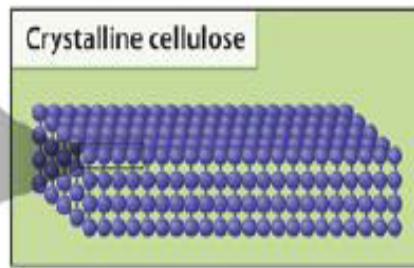
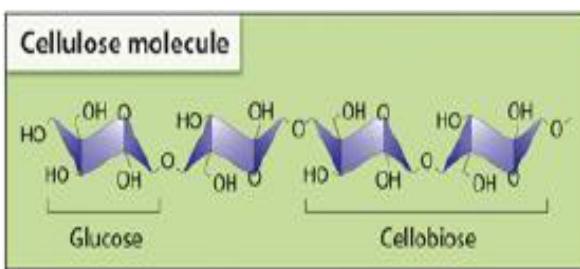
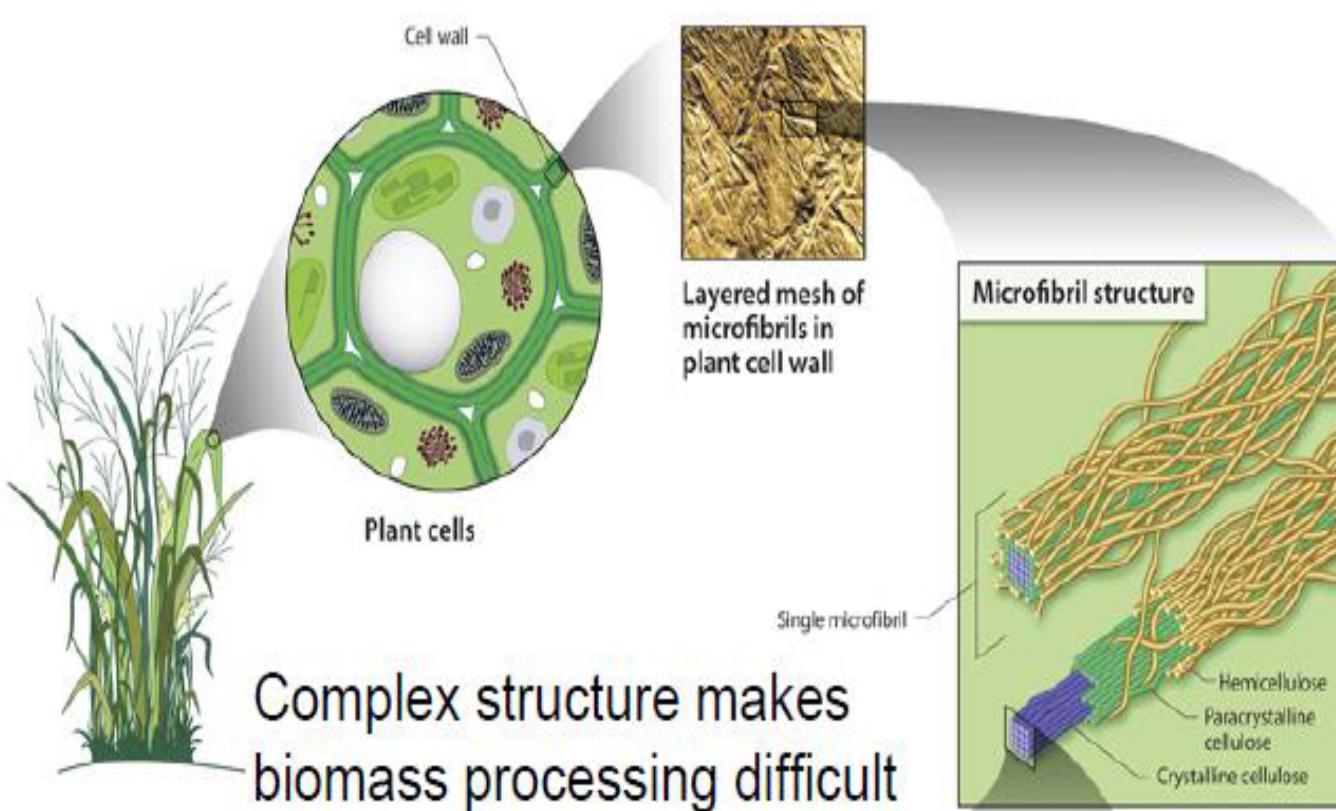
**Epistemic Qs 8.**

**What's the role of evolutionary biology in determining  
the structure of lignocelluloses in plant cell walls?**

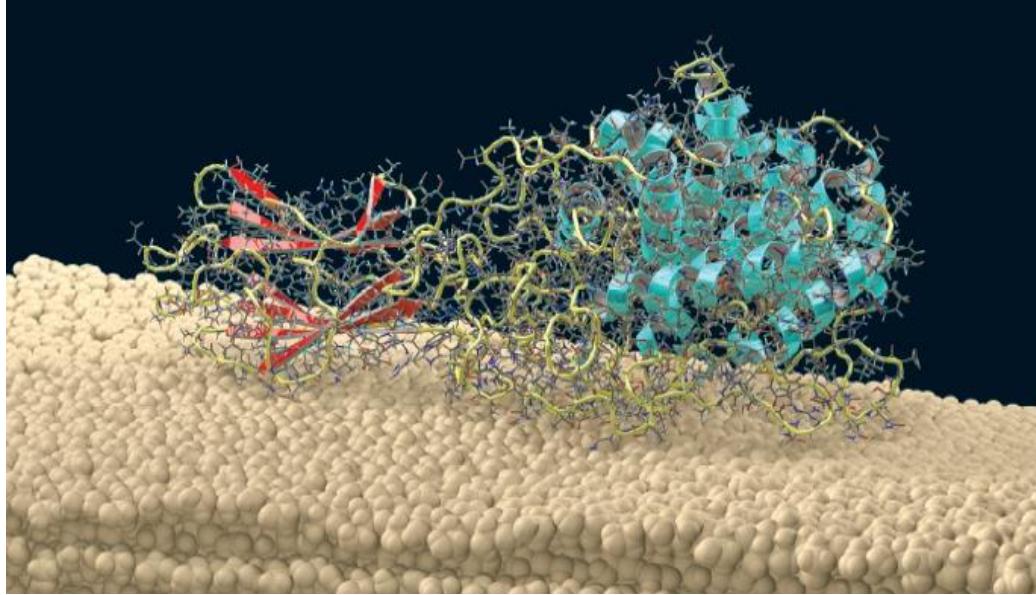
**Epistemic Qs 9.**

**How do enzymes connect to evolutionary biology?**

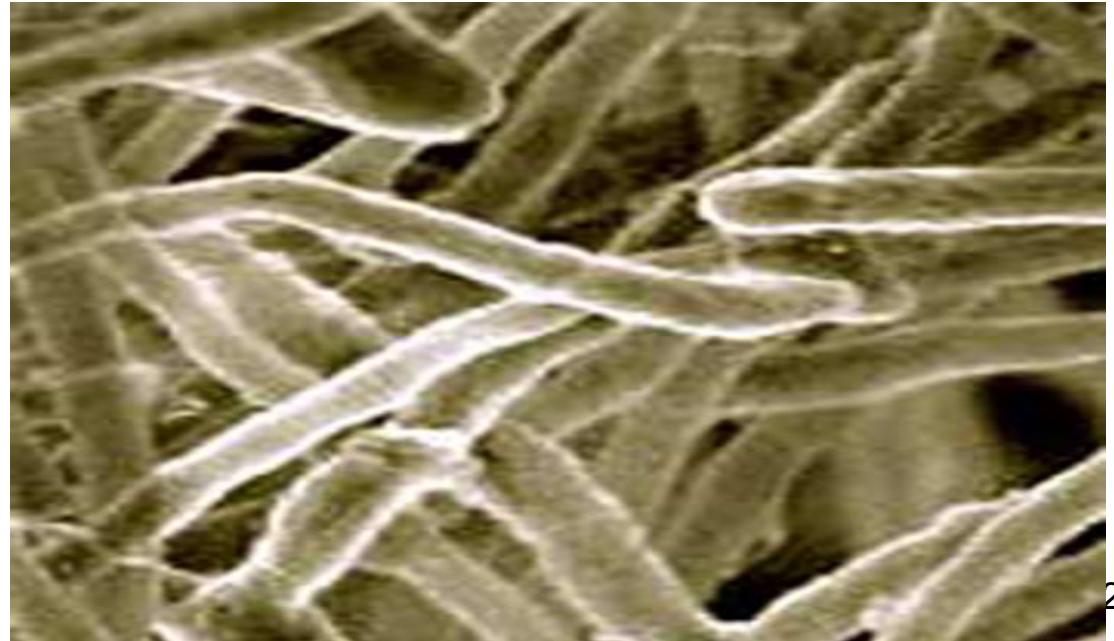
# Complex Biomass Structure



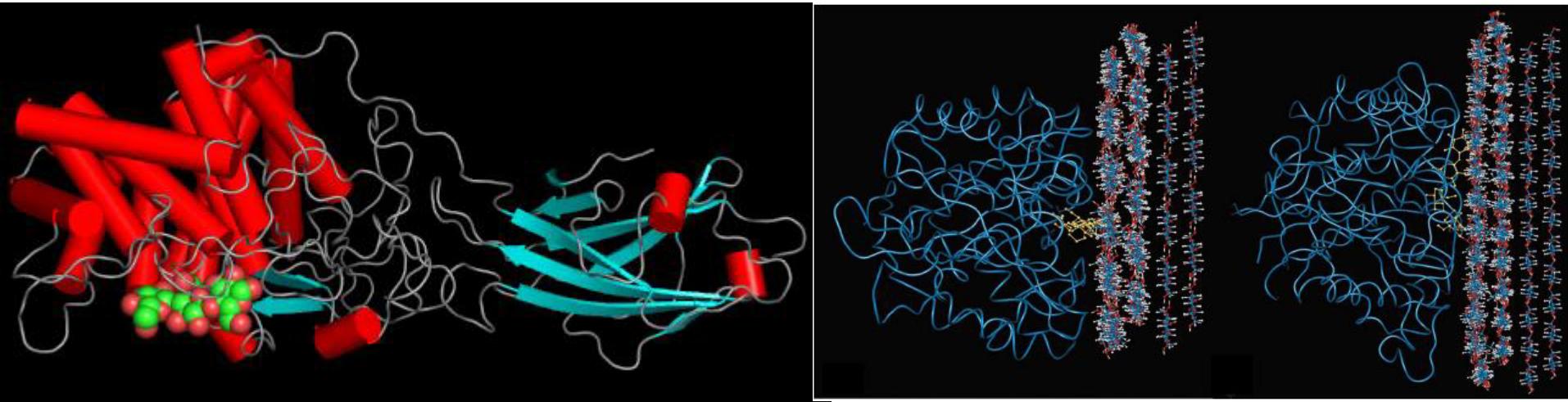
# Cellulase on Cellulose



*Trichoderma reesei* Rut C30  
A mesophilic and filamentous fungus has been used for cellulase enzyme production



# Cellulase



Cellulase is composed of three different type of enzymes:

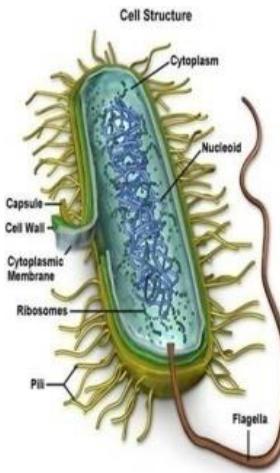
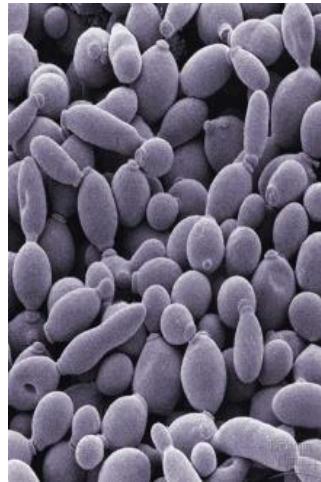
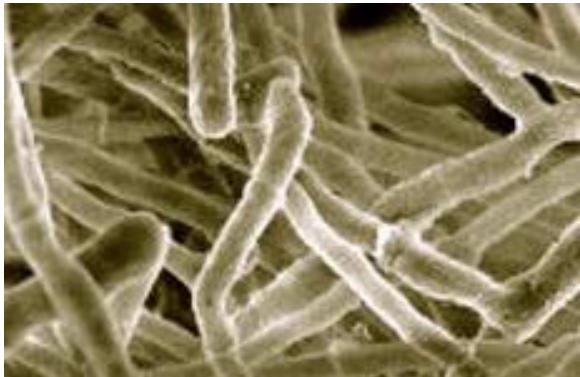
Endo-glucanase

Exo-glucanase (CBH-I and CBH-II)

and

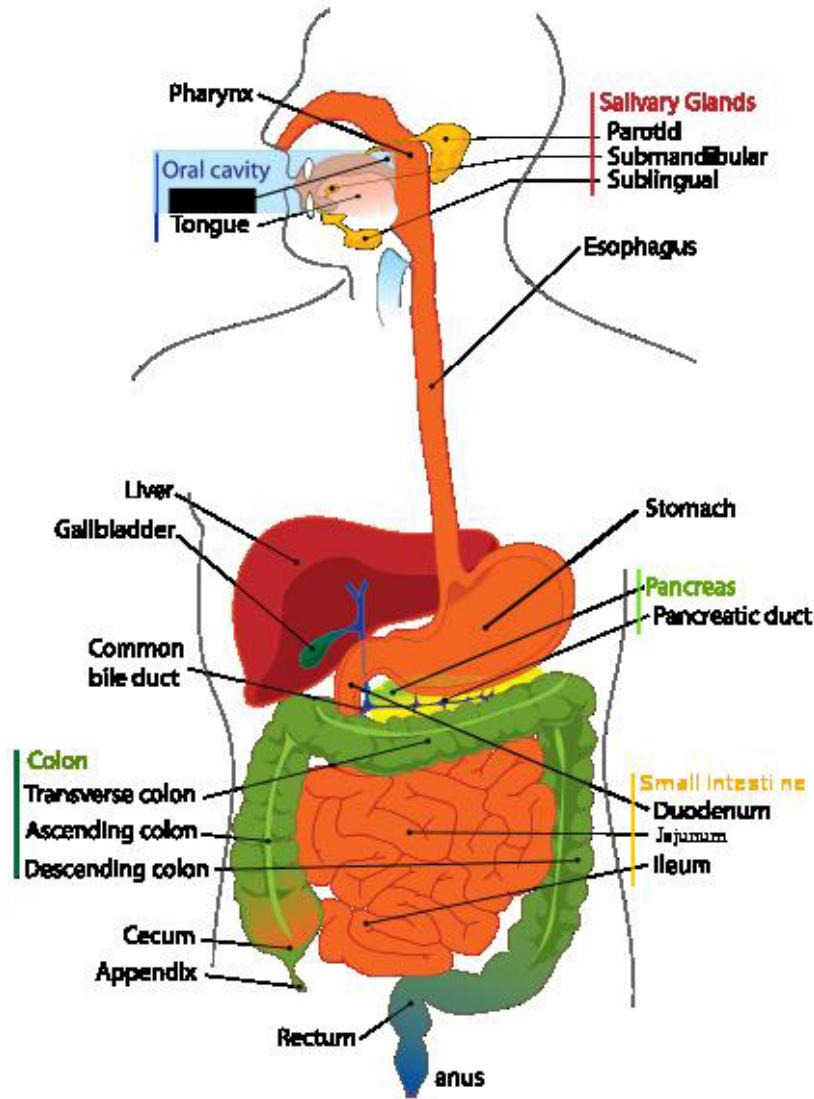
$\beta$ -glucosidase

# Sources of enzymes



- Cellulase obtained from sources such as bacteria, fungi or archaea
- Thermophilic/ Mesophilic cellulase to be important
- Relative concentration of enzyme in the mixture & their kinetic parameters matched to their values in cellulase from *T.reesei* (65-70% CBHI ,10-15% EG) or *T.viride*

# Enzymes are used all over your body!



# Major Digestive Enzymes

Enzyme	Produced In	Site of Release	pH Level
<b>Carbohydrate Digestion:</b>			
Salivary amylase	Salivary Glands	Mouth	Neutral
Pancreatic amylase	Pancreas	Small Intestine	Basic
Maltase	Small intestine	Small intestine	Basic
<b>Protein Digestion:</b>			
Pepsin	Gastric glands	Stomach	Acidic
Trypsin	Pancreas	Small intestine	Basic
Peptidases	Small Intestine	Small intestine	Basic
<b>Nucleic Acid Digestion:</b>			
Nuclease	Pancreas	Small intestine	Basic
Nucleosidases	Pancreas	Small intestine	Basic
<b>Fat Digestion:</b>			
Lipase	Pancreas	Small intestine	Basic

# Enzyme Deficiency

## Protease Deficient:

Cannot digest protein

- causes blood to be more acidic
- Can't produce glucose
- Inadequate hydration in the body

Problems include:

- Arthritis
- Bone spurs
- Hypoglycemia
- Edema (swelling)
- Toxic Colon
- Ear infections in children
- Compromised Immune System
- Predisposed to PMS



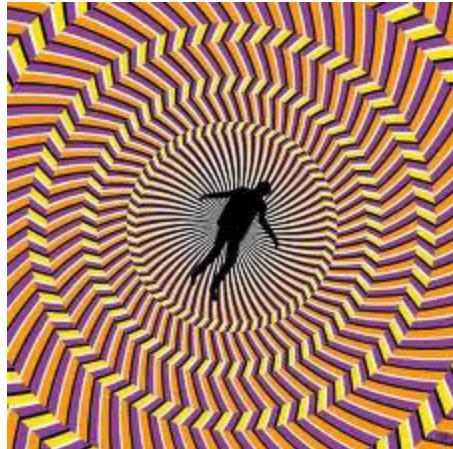
# Enzyme Deficiency

## Lipase Deficient:

Lipase digests fats and fat-soluble vitamins

## Problems include:

- High Cholesterol
- Difficulty losing weight
- High triglycerides
- Decreased cell permeability(can't get glucose out of cells)
- Muscle Spasms
- Chronic Fatigue Syndrome
- Spastic Colon
- Vertigo
- Early Menopause



# Enzyme Deficiency



## Amylase Deficient:

Amylase digests starches and polysaccharides – end result is glucose

Also digests dead white blood cells (pus)

Can be cause by excessive consumption of Carbohydrates

## Problems Include:



- Skin problems: Abscesses, Psoriasis, Eczema, allergic reactions to bee stings
- Lung problems: asthma and emphysema
- Phosphorus deficiency
  - Thick blood
  - Gastritis

# Enzyme Deficiency

## Sucrase Deficient

Cannot split sucrose into glucose

Glucose is basically brain food

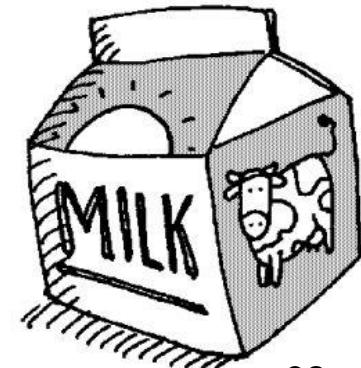
- Problems include: moodiness, depression,
- panic attacks, manic and schizophrenic behavior, severe mood swings and seizures.



## Lactase Deficient

Cannot digest lactose

- Causes cramping and diarrhea



# Flow & Form: Engineering the interactions between Transport and Reactions

**Epistemic Qs 15.**

**How does mixing/transport limitation influence reaction rate (kinetics)?**

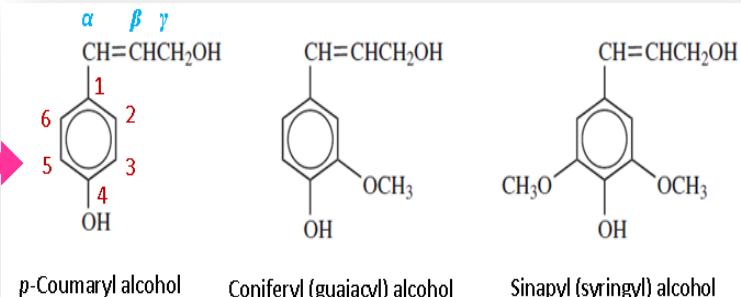
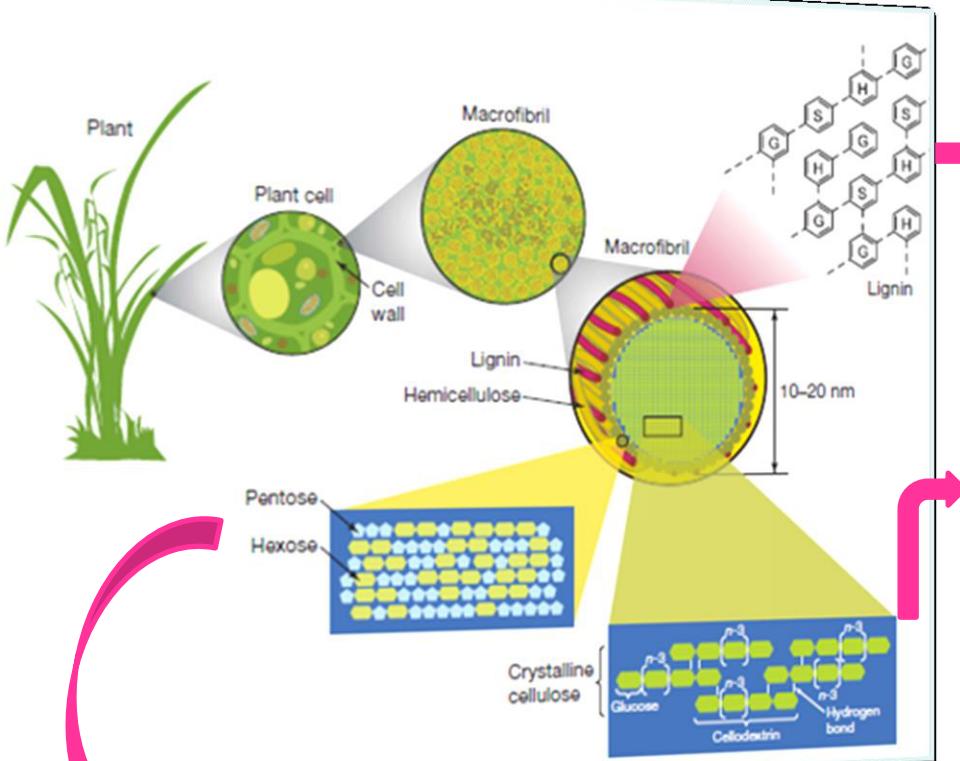
**Epistemic Qs 16.**

**How to manipulate reactor mixing/type to regulate product distribution?**

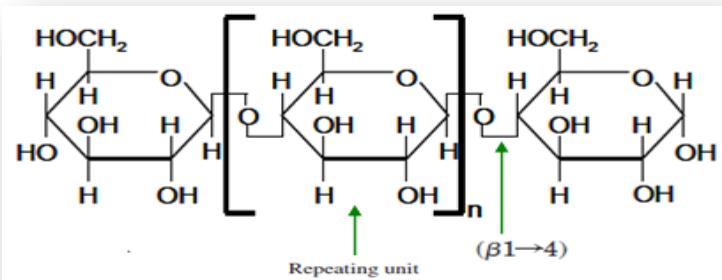
**Epistemic Qs 20.**

**What determines the rate of transport of enzymes to solid substrate surface in a well-mixed reactor?**

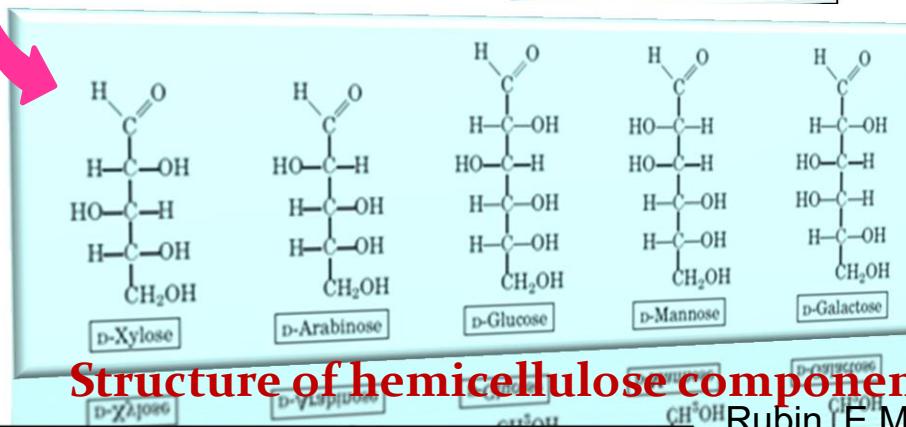
# Structure of Lignocellulosic Biomass



## Structure of lignin components



## Structure of cellulose



## Structure of hemicellulose components

<b>Cellulose</b>	<b>15 - 55%</b>
<b>Hemicelluloses</b>	<b>10 - 50%</b>
<b>Lignin</b>	<b>7 - 35%</b>

Rubin, E.M., 2008. *Nature Rev.*, 454, 841–845.

Jorgensen *et al.*, 2007. *Biofuels, Bioprod. Bioref.* 1, 119–137.

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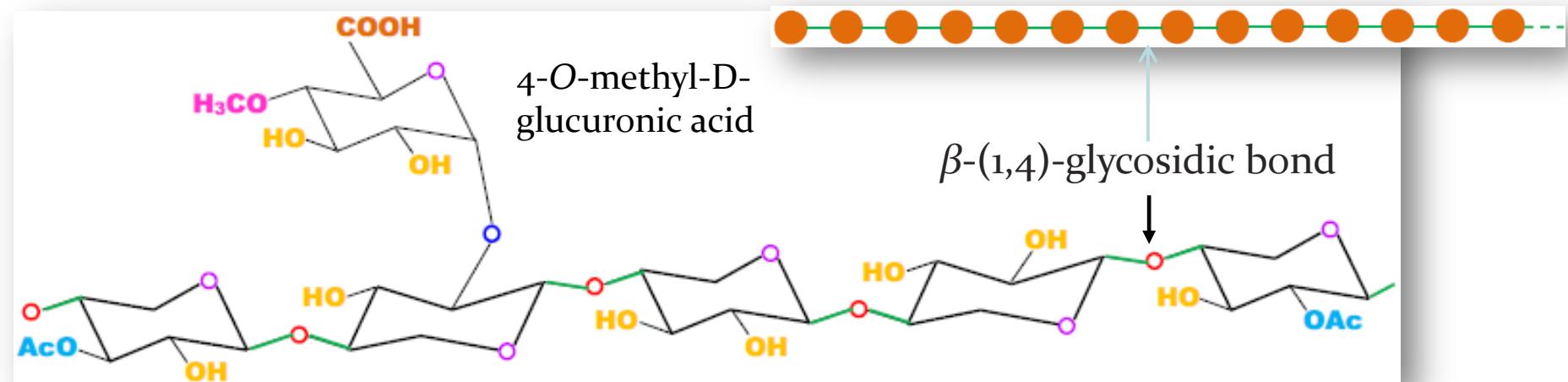
Ebringerova *et al.*, 2005. *Adv. Polym. Sci.* 186, 1–67.

Average value

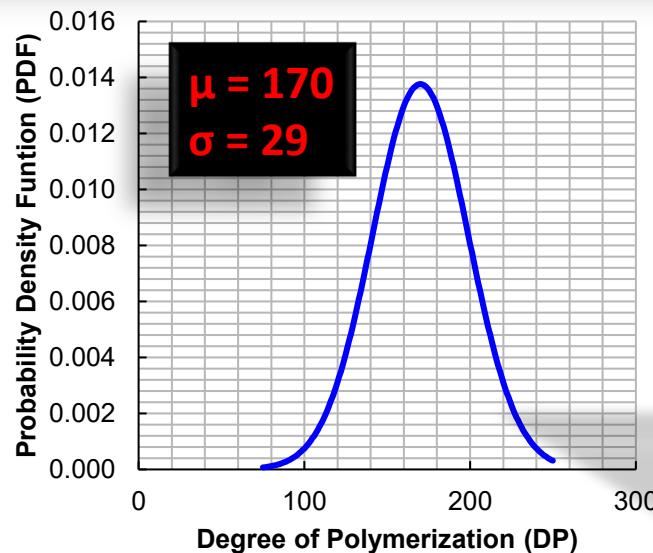
Cellulose : Hemicelluloses = 1.7:1

# Hemicellulose Hydrolysis: at a glance

## Structure of beechwood xylan



Sugar	%
Xylose	90.8
Arabinose	1.1
Glucose	4.7
Uronic acid	3.4

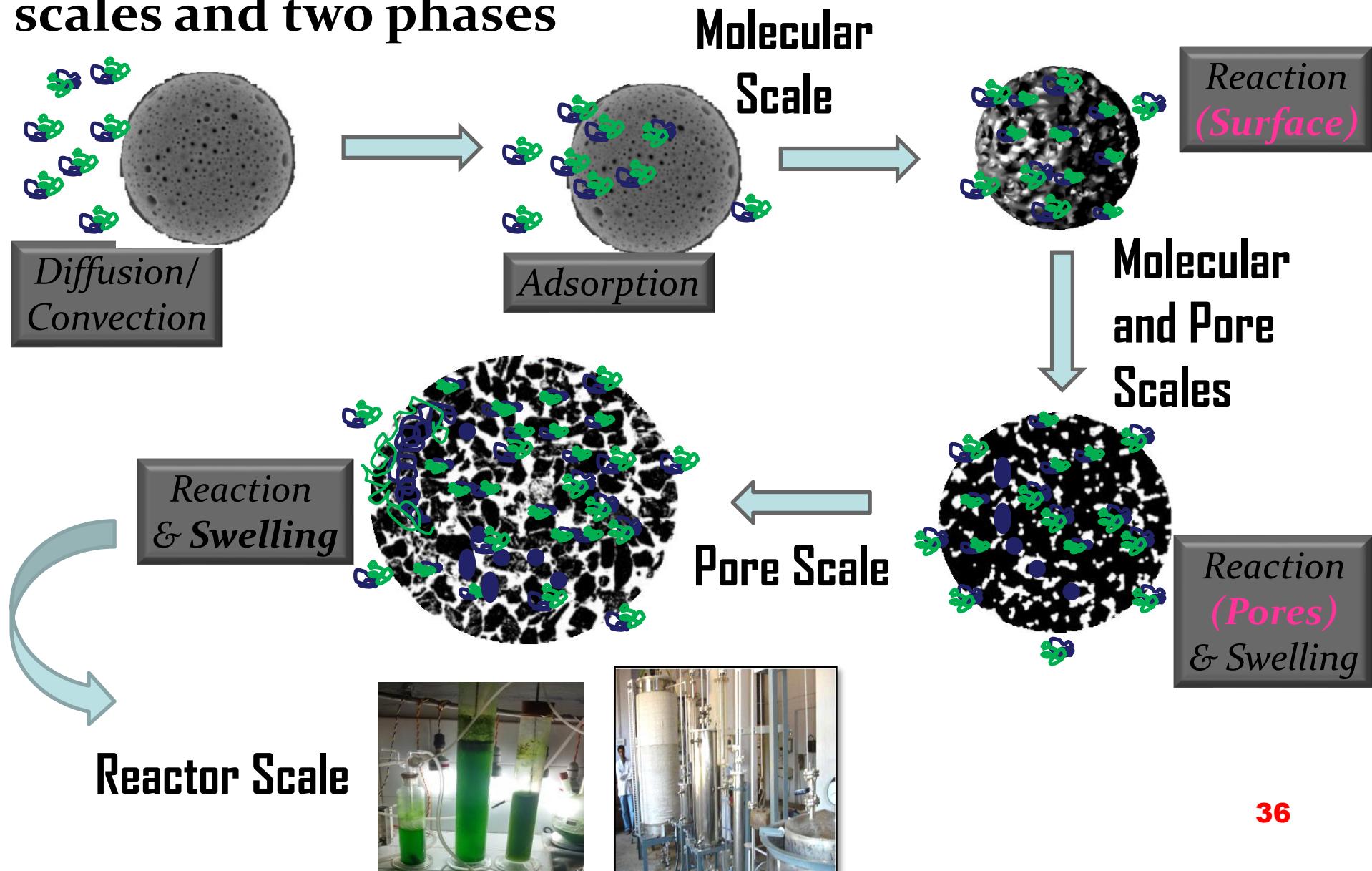


Xylose diameter	0.64 nm
Molecular size of xylan	48 – 160 nm
Average size	109 nm

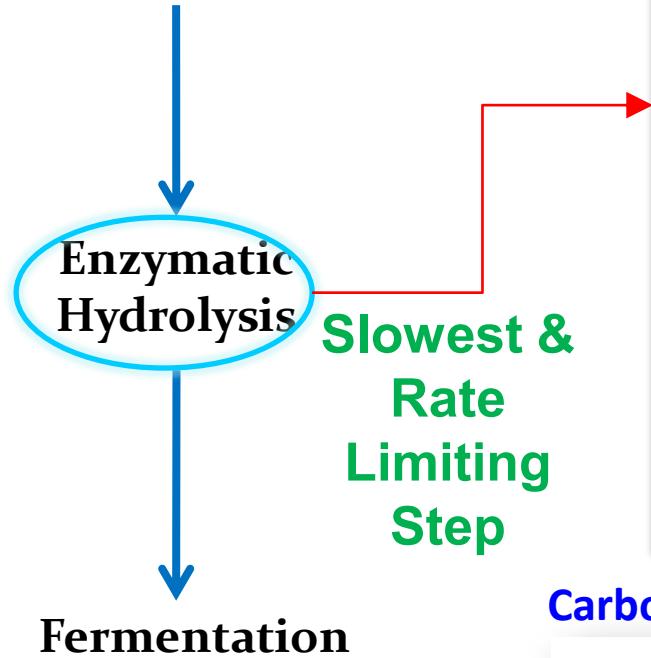
Timell and Syracuse, 1967. *Wood Sci. Technol.* 1, 45–70.

Dusterhiift *et al.*, 1997. *Enzyme Microb. Technol.* 20, 437–445.

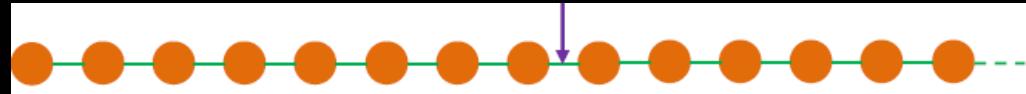
# Complexities of the Multiscale System: coupled Transport and Reaction occur across three scales and two phases



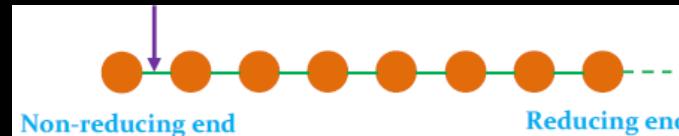
## Pre-treatment of Biomass



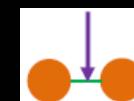
✓ **Endoxylanase** randomly cleaves  $\beta$ -(1 $\rightarrow$ 4) glycosidic bonds



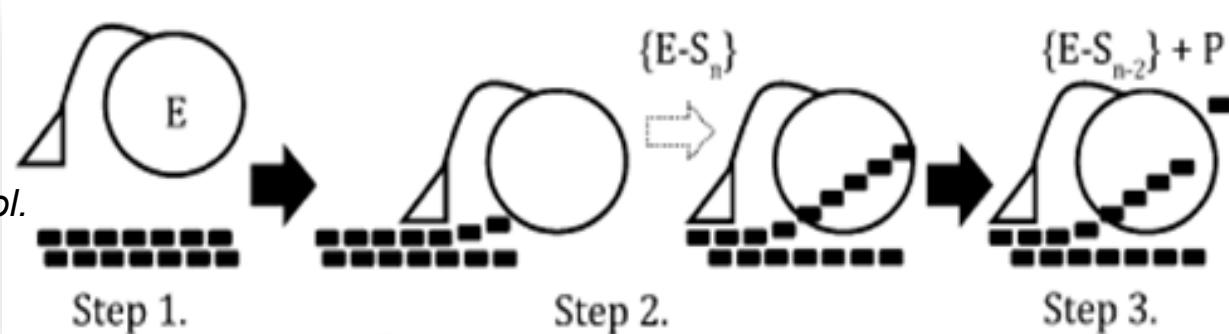
✓ **Exoxyylanase** releases xylose from xylan and xylo-oligosaccharides from *non-reducing ends*



✓  **$\beta$ -xylosidase** cleaves xylobiose and xylo-oligosaccharides from *non-reducing ends*



## Carbohydrate Binding Doman (CBD) & Catalytic Doman (CD)



Bastawde, K.B., 1992. *World J. Microbiol. Biotechnol.* 8, 353–368.

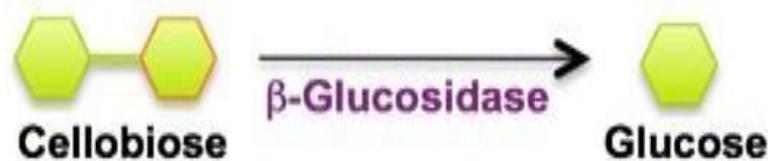
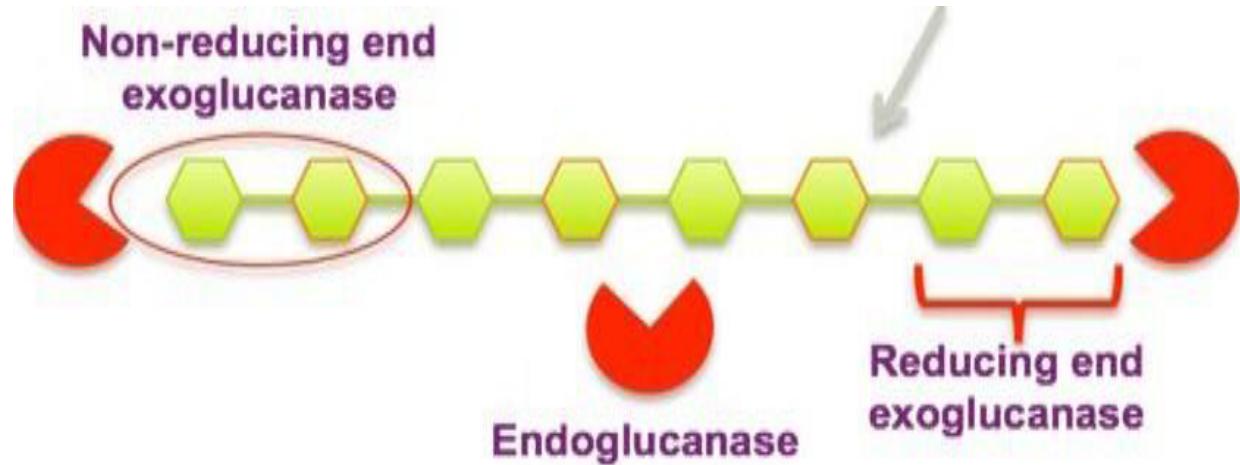
Polizeli *et al.*, 2005. *Appl. Microbiol. Biotechnol.* 67, 577–591.

Gao *et al.*, 2013. *PNAS* 110, 10922–10927.

# Cellulose to Glucose via Enzymatic Hydrolysis Depolymerization

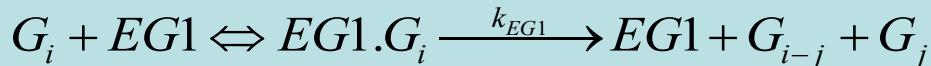
**Substrates**  
**DP= 60-20,000**

PASC DP = 60  
Avicel DP = 300  
Filter Paper DP = 750  
CMC DP = 1500  
Cotton DP = 3000



# Kinetics of Enzymatic Hydrolysis of Cellulose using Cellulase Enzyme

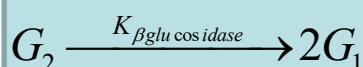
- Enzymes are a part of cellulases obtained from several organisms, including *T.reesei* : Endoglucanase, Exoglucanase &  $\beta$ -glucosidase
  - The endoglucanase cuts the cellulose chain rapidly at random points



- The exoglucanase(CBH) forms complexes with either reducing or the non-reducing end of the cellulose chain cleaving them into primarily cellobiose

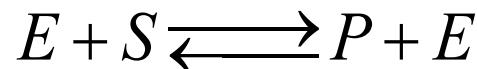


- The  $\beta$ -glucosidase forms glucose by cleaving cellobiose.



# Kinetics of Enzyme Reactions

- The enzymes provides an alternate reaction pathway that produces an activated state of the reactants with lower energy barrier.



- E= enzyme, S= substrate, P= product.
- The enzyme accelerates the reaction but is not consumed in the reaction.
- The enzyme affects the rate of reaction, but does not affect/alter the equilibrium.

# Michaelis-Menten Kinetics

For most enzymes involving single substrates, expts. show that rate of consumption of substrate is given by,

$R_S$  = Rate of disappearance of substrate/reactant

$K_M$  = Michaelis constant =  $C_S$  at which,  $R_S = R_{\max} / 2$

$R_{\max}$  =Maxm. reaction. rate

case 1.

## *Invertase-catalyzed hydrolysis of sucrose into glucose and fructose.*

## *Leonor Micahelis & Maud Menten, 1913*

case2.

# **Derivation fo M-M Kinetics: Reversibility, Parameter Lumping, Asymptotic Solutions**

**Epistemic Qs 4.**

**What's the difference between reversible and irreversible reactions?**

**Epistemic Qs 11.**

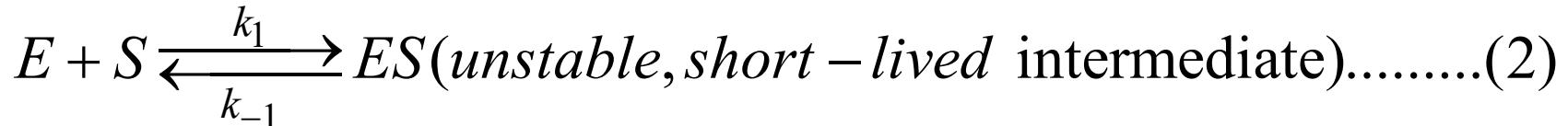
**Why lumped kinetics is more advantageous than detailed kinetics?**

**Epistemic Qs 12.**

**What are the uses of asymptotic solutions to equations?**

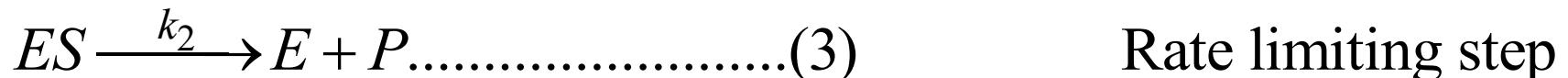
# Derivation of Michaelis-Menten Kinetics

- Enzyme (E) reacts with substrate (S) to form a complex (ES)



Lois Generales de l'Action des Diastases, **Victor Henri, 1903**

In the second step this unstable , the short-lived intermediate complex dissociates yielding the enzyme(E) and the product(P).



Rate of disappearance of substrate S =

$$-R_S = \frac{dC_S}{dt} = -k_1 C_S C_E + k_{-1} C_{ES} \text{.....(4)}$$

# Derivation of Michaelis-Menten Kinetics...Contd

## Rate of formation of 'ES'=

As enzyme is not consumed,

total enzyme = free enzyme + enzyme in complex (ES) form

## Constraint eqn.

# Derivation of Michaelis-Menten Kinetics...Contd

## Rate of product formation =

## Key Assumption:

Quasy steady state(Complex(ES)is unstable breaks down rapidly, its rate of accumulation is zero)

# Derivation of Michaelis-Menten Kinetics...Contd

Substituting eqn 9 into eqn.6

# Derivation of Michaelis-Menten Kinetics... Evaluating the Rxn. Rate in terms of known variables

Substituting eqn 10 & 11 into eqn.4

$$\begin{aligned}
-R_S &= \frac{dC_S}{dt} = -k_1 C_S C_E + k_{-1} C_{ES} \\
&= \frac{k_1 k_2 C_S C_{E0}}{(k_{-1} + k_2) + k_1 C_S} \\
&= \frac{k_2 C_S C_{E0}}{\frac{(k_{-1} + k_2)}{k_1} + C_S} \dots \dots \dots \quad (12)
\end{aligned}$$

# Derivation of Michaelis-Menten Kinetics...Evaluating Michaelis Constants

Comparing:

$$R_S = \frac{R_{\max} C_S}{K_M + C_S}$$

with

$$R_S = \frac{k_2 C_S C_{E0}}{\frac{(k_{-1} + k_2)}{k_1} + C_S}$$

$$\left\{ \begin{array}{l} R_{\max} = k_2 C_{E0} \\ K_M = \frac{(k_{-1} + k_2)}{k_1} \end{array} \right\}$$

$$\left\{ \begin{array}{l} R_{\max} = k_2 C_{E0} \\ K_M = \frac{(k_{-1} + k_2)}{k_1} \end{array} \right\}$$

case1.

$C_S \gg K_M$ ,

$R_S = R_{\max}$

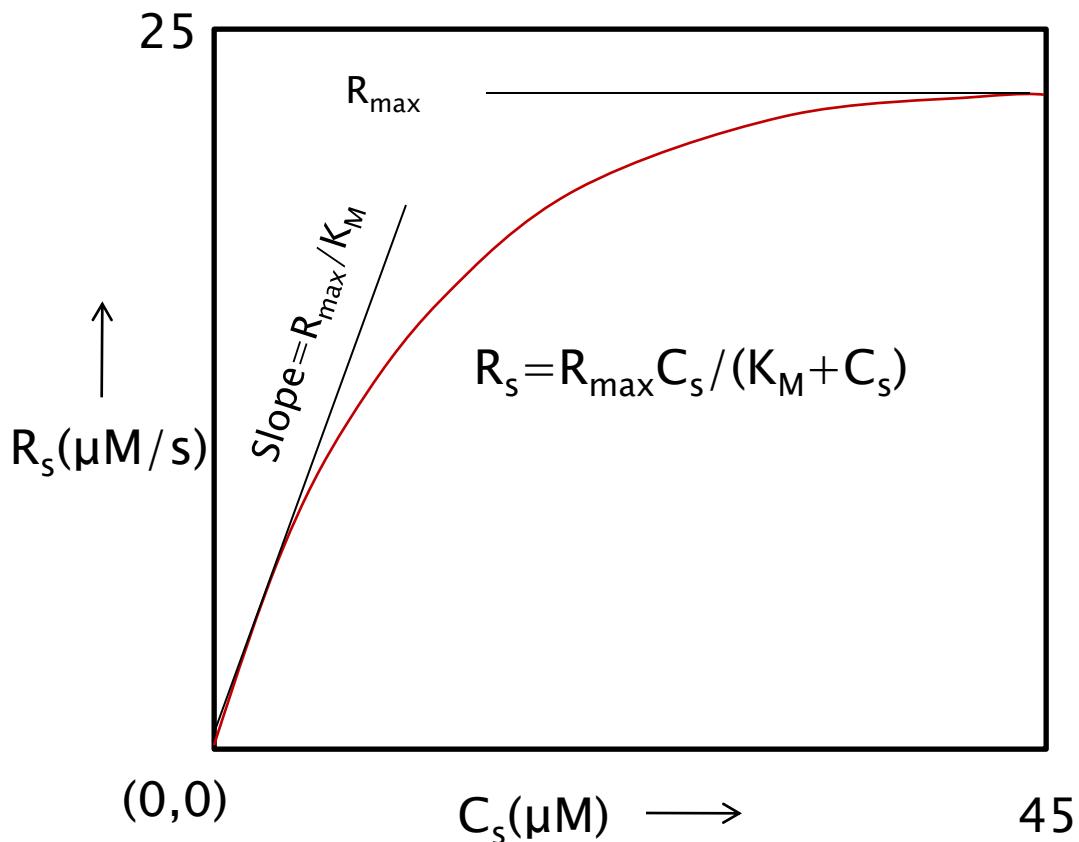
(reaction is '0'th order)

case2.

$C_S \ll K_M$ ,

$$R_S = \frac{R_{\max} C_S}{K_M}$$

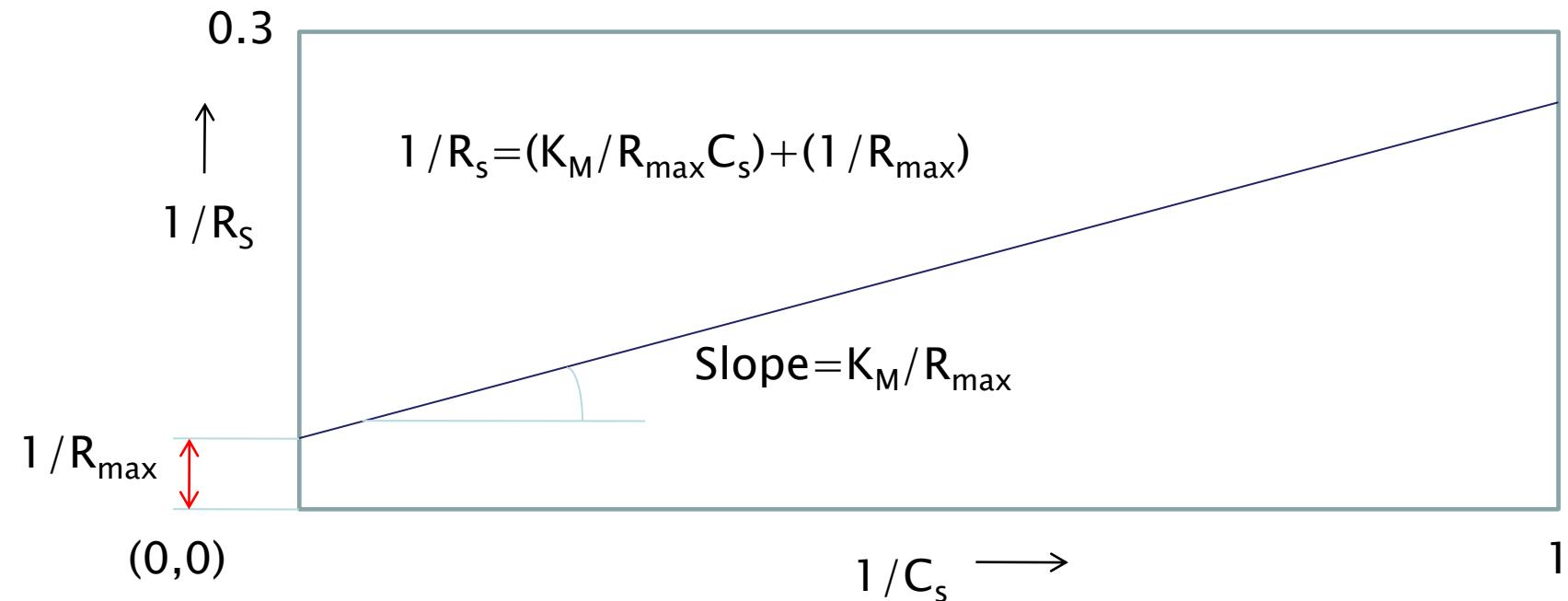
(reaction is 1st order)



## Evaluation of $R_{max}$ & $K_M$

## Lineweaver–Bark Equation

plot  $\frac{1}{R_S}$  (y axis) vs.  $\frac{1}{C_S}$  (x-axis) is a straight line. intercept on y-axis =  $\frac{1}{R_{\max}}$ .



## Evaluation of $R_{max}$ & $K_M$ (contd..)

## Two other important plots.

## **States of a Reactor: Steady, Unsteady and Quasi-steady**

**Epistemic Qs 10.**

**What kind of reactors can attain steady state?**

**Epistemic Qs 13.**

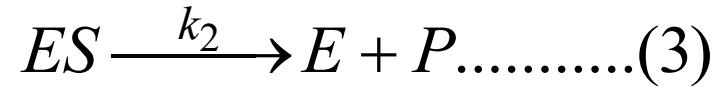
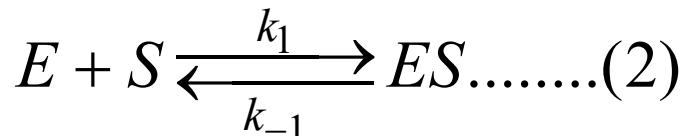
**When is Quasi-steady state (QSS) assumption valid for a species (or several species) participating in a reaction network?**

# When is the Quasi-Steady State Assumption Valid?

## Assumptions:

1. Fast step corresponding to initial formation of ES complex.
  2. Quasi-steady state Complex (ES) is unstable, breaks down rapidly, its rate of accumulation is zero

$$\frac{dC_{ES}}{dt} \approx 0$$



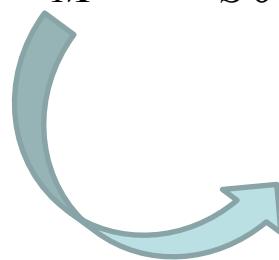
In this period, assume

# When is the Quasi-Steady State Assumption Valid?...Contd.

## Balance Equation for unstable complex ES:

solving eqn. (17) with initial condition at  $t=0$ ,  $C_{ES}=0$ .

$$C_{ES} = \frac{C_{S0}C_{E0}}{K_M + C_{S0}} \left\{ 1 - \exp(-k_1(K_M + C_{S0})t) \right\} \dots \dots \dots (18)$$



## Rate constant for temporal variation of $C_{ES}$

Rate constant for first phase (step 'a') =  $k_1(K_M + C_{S0})$

For second phase, (step 'b')

$$\frac{dC_S}{dt} = -\frac{k_2 C_{E0} C_S}{K_M + C_{S0}}$$

Rate constant of this process during initiation =  $\frac{k_2 C_{E0}}{K_M + C_{S0}}$

For quasi steady state assumption to be valid:

Step 'a' must be much faster than Step 'b'.

$\therefore$  Rate constant for step (b)  $\ll$  rate constant for step (a)

$$\text{or, } \frac{k_2 C_{E0}}{K_M + C_{S0}} \ll k_1(K_M + C_{S0})$$

$$\text{or, } \frac{C_{E0}}{K_M + C_{S0}} \ll (1 + \frac{k_{-1}}{k_2})(1 + \frac{C_{S0}}{K_M})$$

## Regulation of enzyme activity

- Need for inhibition: To control the amount of product formed by rxn. Because
  1. Product may stimulate a biochemical pathway that is needed at certain times (e.g. growth).
  2. Product may be biologically active over a narrow concentration range.
  3. Excess accumulation of products may require too much energy or interfere with other pathway.

# Inhibition

*How does one inhibit?*

by regulating substrate, products or other molecules that interact with the enzymes.

*What does regulation lead to?*

- a. inhibition (intrinsic as well as extrinsic)
- b. activation (extrinsic)

*What are the types of inhibition?*

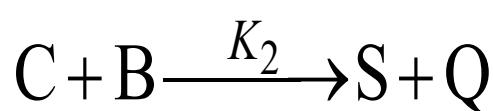
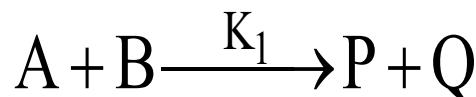
- 1. Competitive inhibition
- 2. a. Un-competitive inhibition
  - b. Non-competitive inhibition
- 3. Substrate inhibition.

# Epistemic Qs 14.

## Reaction networks: series/parallel/series-parallel?

### Examples of competitive Reactions

- Competitive parallel :  $(A:B)_{\text{fed}} = 1:1$

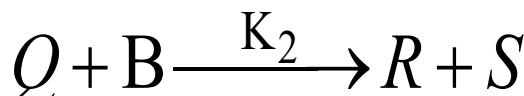
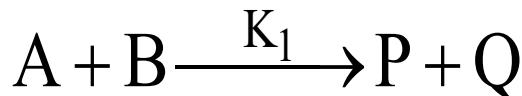


S:desired product

Case 1:  $K_2 \gg K_1$  no P

Case 2:  $K_1 \gg K_2$  no S

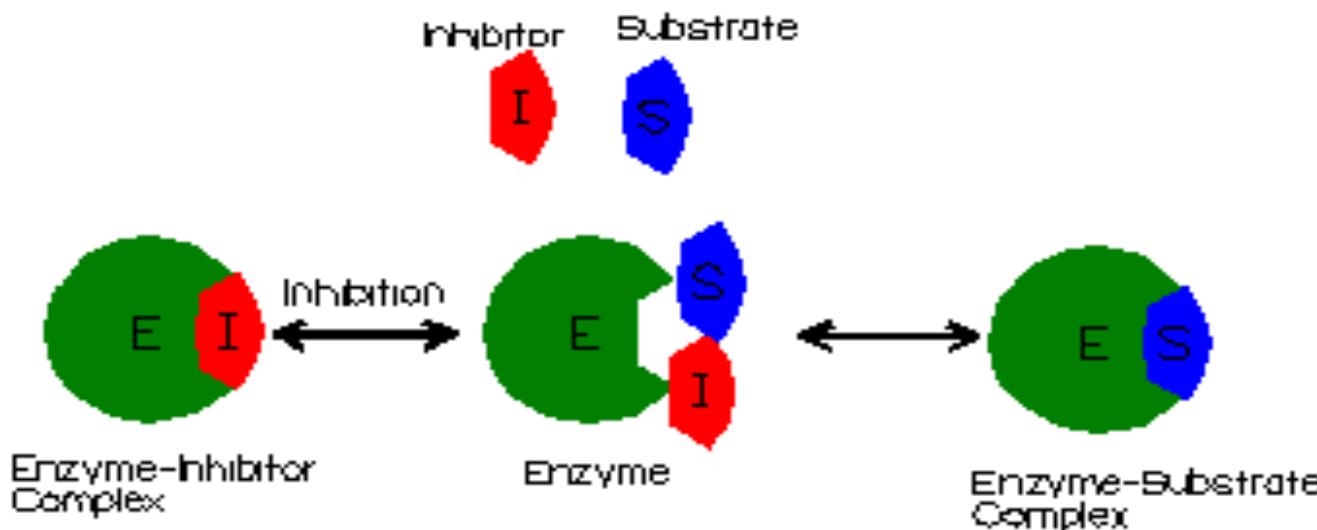
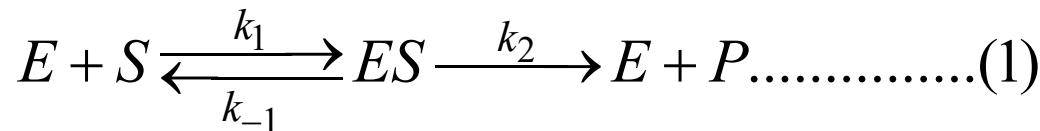
- Competitive Consecutive:  $(A:B)_{\text{fed}} = 1:1$



If  $\frac{K_1}{K_2} \rightarrow \infty$ , no S is formed.

## Competitive inhibition

Inhibitor (I) blocks active sites of enzymes



# Derivation

## Assumptions:

## 1. Quasi-steady state for ES

$$\frac{dC_{ES}}{dt} \approx 0$$

$$or, k_1 C_S C_E - (k_{-1} + k_2) C_{ES} = 0$$

## 2. Reaction 2 attains equilibrium

$$k_i C_E C_I = k_{-i} C_{EI}$$

or,  $K_I = \frac{k_{-i}}{k_i} = \frac{C_E C_I}{C_{EI}}$  .....(4)

## 3. Constraint equation:

$$C_{E0} = C_{ES} + C_E + C_{EI}$$
$$= \frac{C_E C_S}{K_M} + C_E + \frac{C_E C_I}{K_I} .....(5)$$

or,  $C_E = \frac{C_{E0}}{\frac{C_S}{K_M} + 1 + \frac{C_I}{K_I}}$  .....(6)

## Competitive inhibition (contd..)

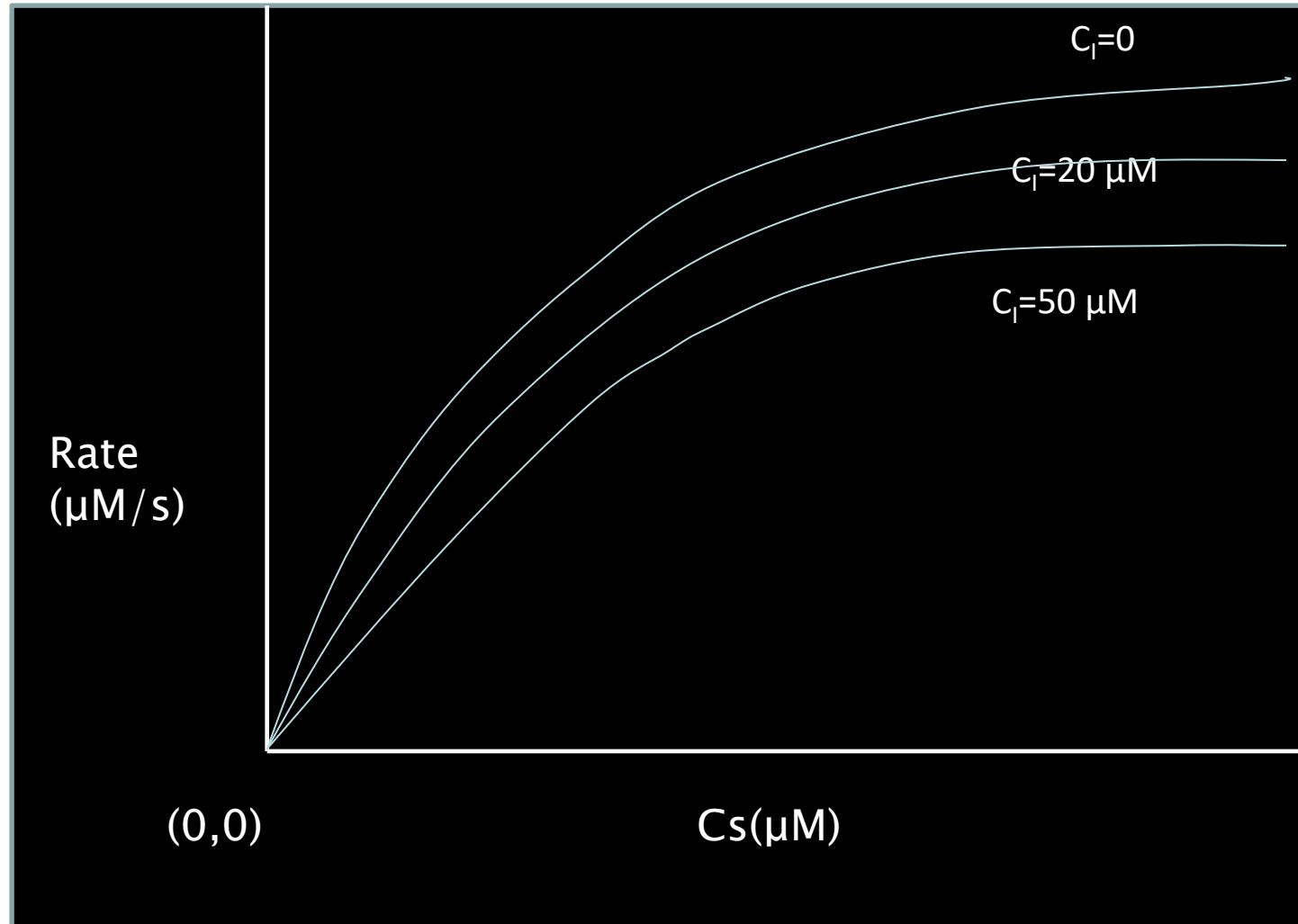
Substituting eqn. (6) in eqn(3):

$$C_{ES} = \frac{C_E C_S}{K_M} = \frac{C_{E0} C_S}{K_M \left(1 + \frac{C_I}{K_I}\right) + C_S} \dots \dots \dots (7)$$

*where,*

$$R_{\max} = k_2 C_{E0} \quad \& \quad \bar{K}_M = K_M \left(1 + \frac{C_I}{K_I}\right)$$

since,  $C_J, K_J > 0, \bar{K}_M > K_M$ .

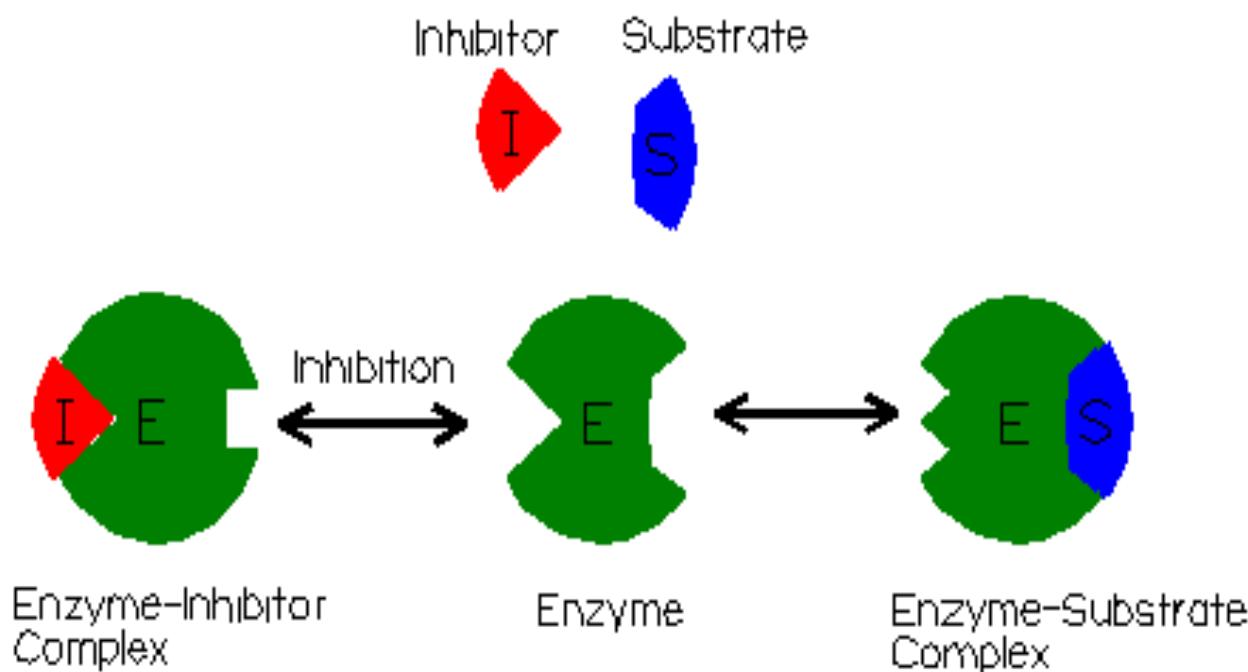


At  $C_s \longrightarrow 0$ , slope =  $\frac{R_{\max}}{K_M}$

Note: as  $K_M$  decrease, slopes increase

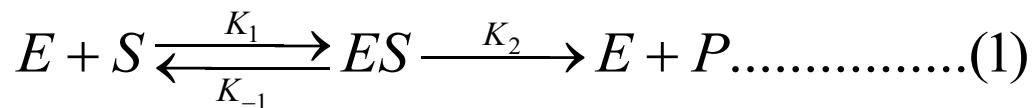
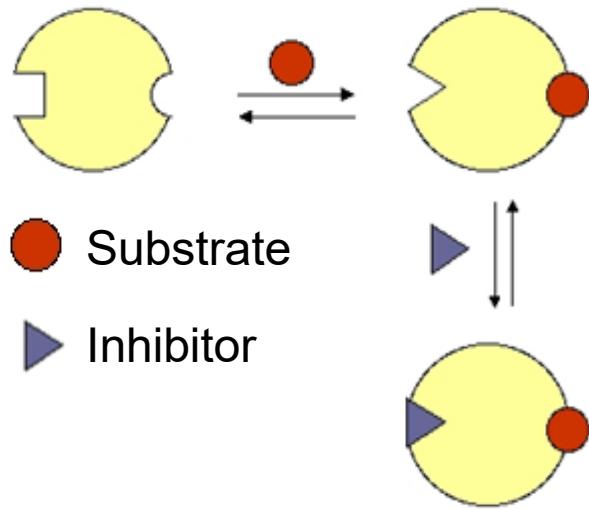
# Uncompetitive Inhibition

Instead of binding to the active sites inhibition can bind to other sites on the enzymes, reducing the reaction rate.



## Uncompetitive inhibition

- It does not directly block active sites of enzymes but interacts with enzyme bounded substrate on another sites, reducing reaction rate.



1. Assumption: Reaction (2) is in equilibrium:

## 2. Constraint eqn :

### 3. Quasi-steady state assumption for 'ES'

$$\frac{dC_{ES}}{dt} = K_1 C_E C_S - K_{-1} C_{ES} - K_2 C_{ES} - \cancel{K_3 C_{ES} C_I} + \cancel{K_4 C_{ESI}} = 0$$

$$(K_{-1} + K_2)C_{ES} = K_1 C_E C_S$$

## Uncompetitive Inhibition (Contd....)

Substituting (3) & (5) in (4)

$$C_{EO} = C_E \left( 1 + \frac{K_1}{K_{-1} + K_2} C_S + \frac{K_1}{K_{-1} + K_2} C_S \frac{C_I}{K_I} \right);$$

$$C_E = \frac{C_{EO}}{1 + \frac{C_S}{K_M} + \frac{C_S}{K_M} \frac{C_I}{K_I}}$$

$$C_{ES} = \frac{C_E C_S}{K_M} = \frac{C_{EO} C_S}{K_M + C_S \left( 1 + \frac{C_I}{K_I} \right)}$$

# Uncompetitive Inhibition (Contd....)

$$Rate = \frac{dc_p}{dt} = K_2 C_{ES} = \frac{K_2 C_{EO} C_S}{K_M + \left(1 + \frac{C_I}{K_I}\right) C_S}$$

$$= \frac{\frac{K_2 C_{EO}}{C_I / K_I} C_S}{\frac{K_M}{C_I / K_I} + C_S} = \frac{\tilde{R}_{\max} C_S}{\tilde{K}_M + C_S}$$

$$\tilde{K}_M = \frac{K_M}{1 + \frac{C_I}{K_I}}$$

$$\tilde{R}_{\max} = \frac{K_2 C_{EO}}{1 + \frac{C_I}{K_I}}$$

## Uncompetitive Inhibition (Contd....)

$$\tilde{K}_M = \frac{K_M}{1 + \frac{C_I}{K_I}}$$

$$\tilde{R}_{\max} = \frac{K_2 C_{EO}}{1 + \frac{C_I}{K_I}}$$

$$C_I, K_I > 0,$$

$$\tilde{K}_M < K_M, \tilde{R}_{\max} < R_{\max}$$

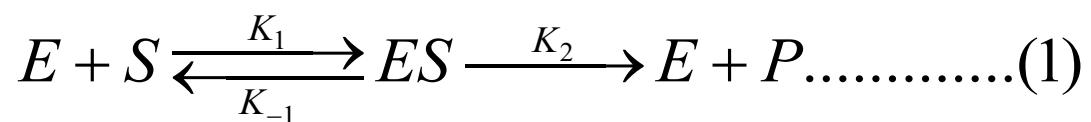
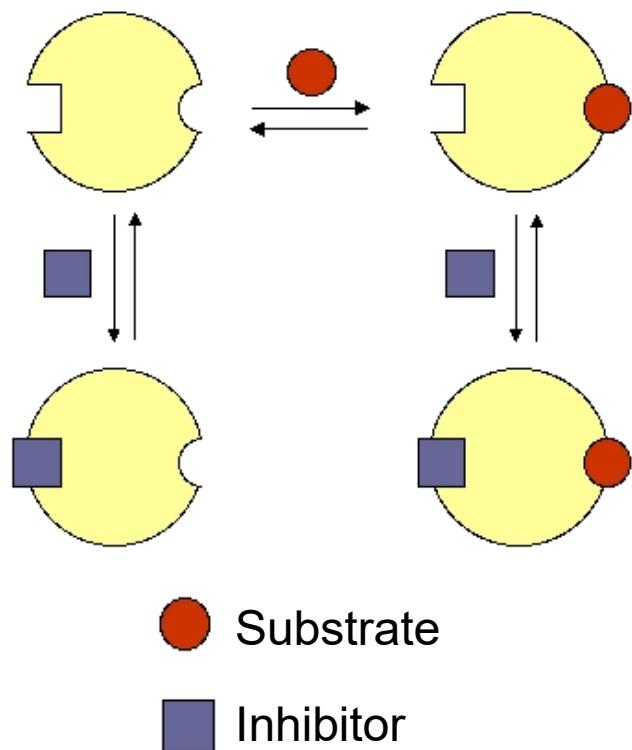
$$\text{However, } \frac{\tilde{R}_{\max}}{\tilde{K}_M} = \frac{K_2 C_{EO}}{K_M} = \frac{R_{\max}}{K_M}$$

For,  $C_S \rightarrow 0$ , slope remains unchanged;

However maxm. rxn. rate  $R_{\max}$  *decreases*.

## Non-competitive inhibition

Both the active sites as well as non-active sites are blocked by the inhibitor, thus reducing the reaction rate.



1. Assumption: reactions (2) and (3) are in equilibria:

where,  $K_I^{EI} = \frac{K_{-i}^{EI}}{K_i^{EI}}$  &  $K_I^{ESI} = \frac{K_{-i}^{ESI}}{K_i^{ESI}}$

## 2. Quasi Steady-state assumption for 'ES'

$$\begin{aligned} \frac{dC_{ES}}{dt} &= K_1 C_E C_S - K_{-1} C_{ES} - K_2 C_{ES} - \cancel{K_i^{ESI} C_{ES} C_I} + \cancel{K_i^{ESI} C_{ESI}} = 0 \\ \Rightarrow (K_{-1} + K_2) C_{ES} &= K_1 C_E C_S \Rightarrow C_{ES} = \frac{K_1}{K_{-1} + K_2} C_E C_S = \frac{C_E C_S}{K_M} \dots\dots\dots (6) \end{aligned}$$

## Non-competitive Inhibition(contd...)

3. Constraint eqn :  $C_{EO} = C_E + C_{ES} + C_{EI} + C_{ESI}$

$$\begin{aligned} C_{EO} &= C_E + \frac{C_E C_S}{K_M} + \frac{C_E C_I}{K_I^{EI}} + \frac{C_{ES} C_I}{K_I^{ESI}} \\ &= C_E + \frac{C_E C_S}{K_M} + \frac{C_E C_I}{K_I^{EI}} + \frac{C_E C_S}{K_M} \frac{C_I}{K_I^{ESI}} \end{aligned}$$

or,

$$C_E = \frac{C_{EO}}{1 + \frac{C_S}{K_M} + \frac{C_I}{K_I^{EI}} + \frac{C_S}{K_M} \frac{C_I}{K_I^{ESI}}}$$

## Non-competitive Inhibition (contd...)

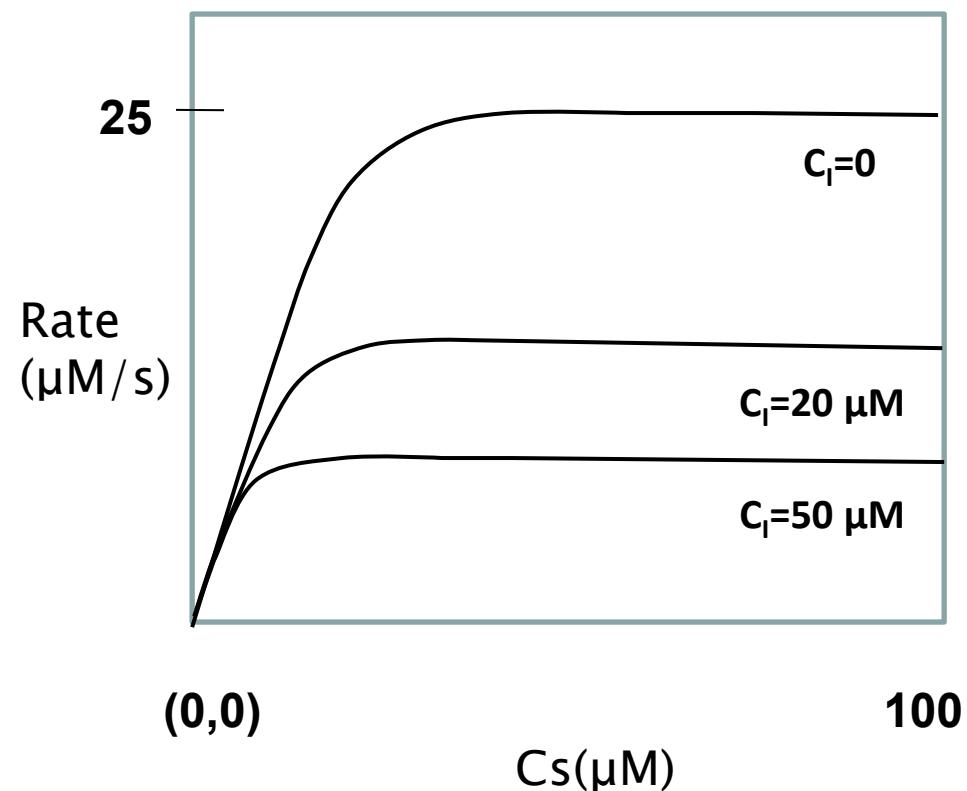
$$Rate = \frac{dc_p}{dt} = K_2 C_{ES} = K_2 \frac{C_E C_S}{K_M} = \frac{K_2 C_{EO} C_S}{K_M \left[ 1 + \frac{C_S}{K_M} + \frac{C_I}{K_I^{EI}} + \frac{C_S}{K_M} \frac{C_I}{K_I^{ESI}} \right]}$$

$$= \frac{K_2 C_{EO} C_S}{\left[ K_M + \frac{C_I K_M}{K_I^{EI}} + C_S \left( 1 + \frac{C_I}{K_I^{ESI}} \right) \right]} = \frac{\tilde{R}_{\max} C_S}{\tilde{K}_M + C_S}$$

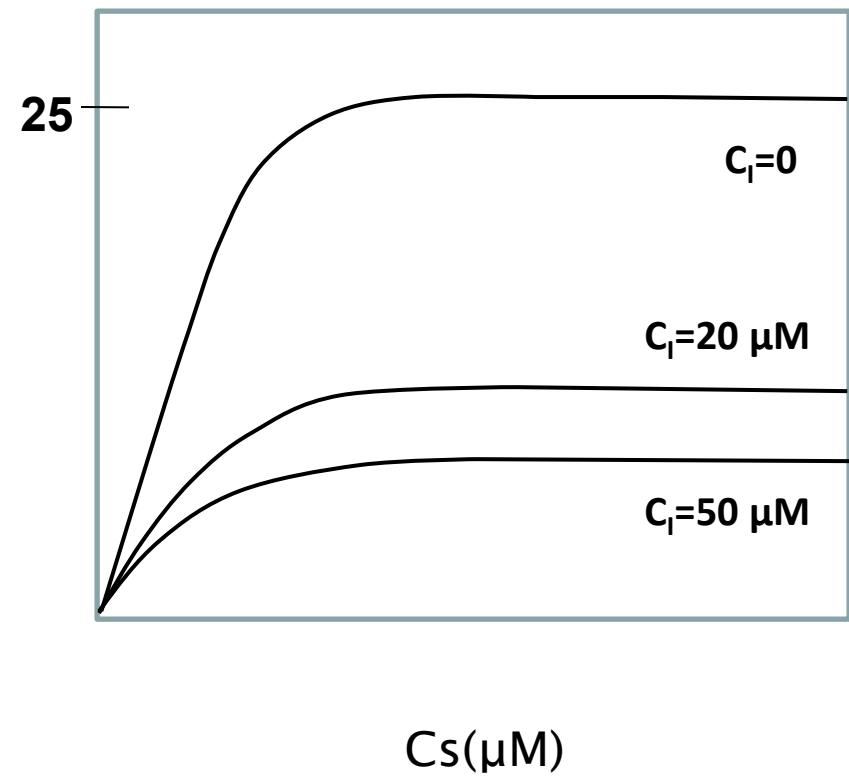
$$\tilde{R}_{\max} = \frac{K_2 C_{EO}}{\left( 1 + \frac{C_I}{K_I^{ESI}} \right)} \quad \& \quad \tilde{K}_M = K_M \frac{\left( 1 + \frac{C_I}{K_I^{EI}} \right)}{\left( 1 + \frac{C_I}{K_I^{ESI}} \right)}$$

$$\begin{aligned}
 R_{\max} &= 25 \mu\text{M}/\text{s} & K_M &= 5 \mu\text{M} \\
 K_I &= 10 \mu\text{M} & K_I^{\text{ESI}} &= K_I^{\text{EI}} = 10 \mu\text{M}
 \end{aligned}$$

Un-competitive



Non-competitive

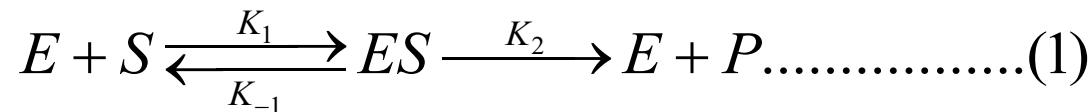


## Epistemic Qs 18:

# How to calculate the maximum substrate loading in Biochemical reactions?

# Substrate inhibition

- Substrate can inhibit the reaction rate by binding to a second site on the enzyme. This second binding does not lead to products formation and is likely to be of lower affinity because it has larger dissociation constant fast binding. Thus, substrate inhibition in many ways is similar to uncompetitive inhibition.



# Substrate Inhibition (Derivation)

1. Assumption: Reaction (2) is in equilibrium:

$$K_{D11} = \frac{K_{-11}}{K_{11}} = \frac{C_{ES}C_S}{C_{ESS}} \quad \Rightarrow C_{ESS} = \frac{C_{ES}C_S}{K_{D11}} \dots \dots \dots (3)$$

## 2. Quasi Steady State Assumption:

Eqn. (3) becomes:

$$C_{ESS} = \frac{C_{ES}C_S}{K_{D11}} = \frac{C_E C_s^2}{K_M K_{D11}} \dots \dots \dots (5)$$

## Substrate Inhibition (Derivation...Contd.)

3. Constraint eqn.:  $C_{EO} = C_E + C_{ES} + C_{ESS}$

$$C_{EO} = C_E \left( 1 + \frac{C_S}{K_M} + \frac{C_S^2}{K_M K_{D11}} \right)$$

$$\Rightarrow C_E = \frac{C_{EO}}{1 + \frac{C_S}{K_M} + \frac{C_S^2}{K_M K_{D11}}}$$

$$Rate = \frac{dc_p}{dt} = K_2 C_{ES} = \frac{K_2 C_{EO} C_S}{K_M + \left( 1 + \frac{C_S}{K_{D11}} \right) C_S}$$

# Substrate Inhibition (Derivation: Maxim Rexn. Rate)

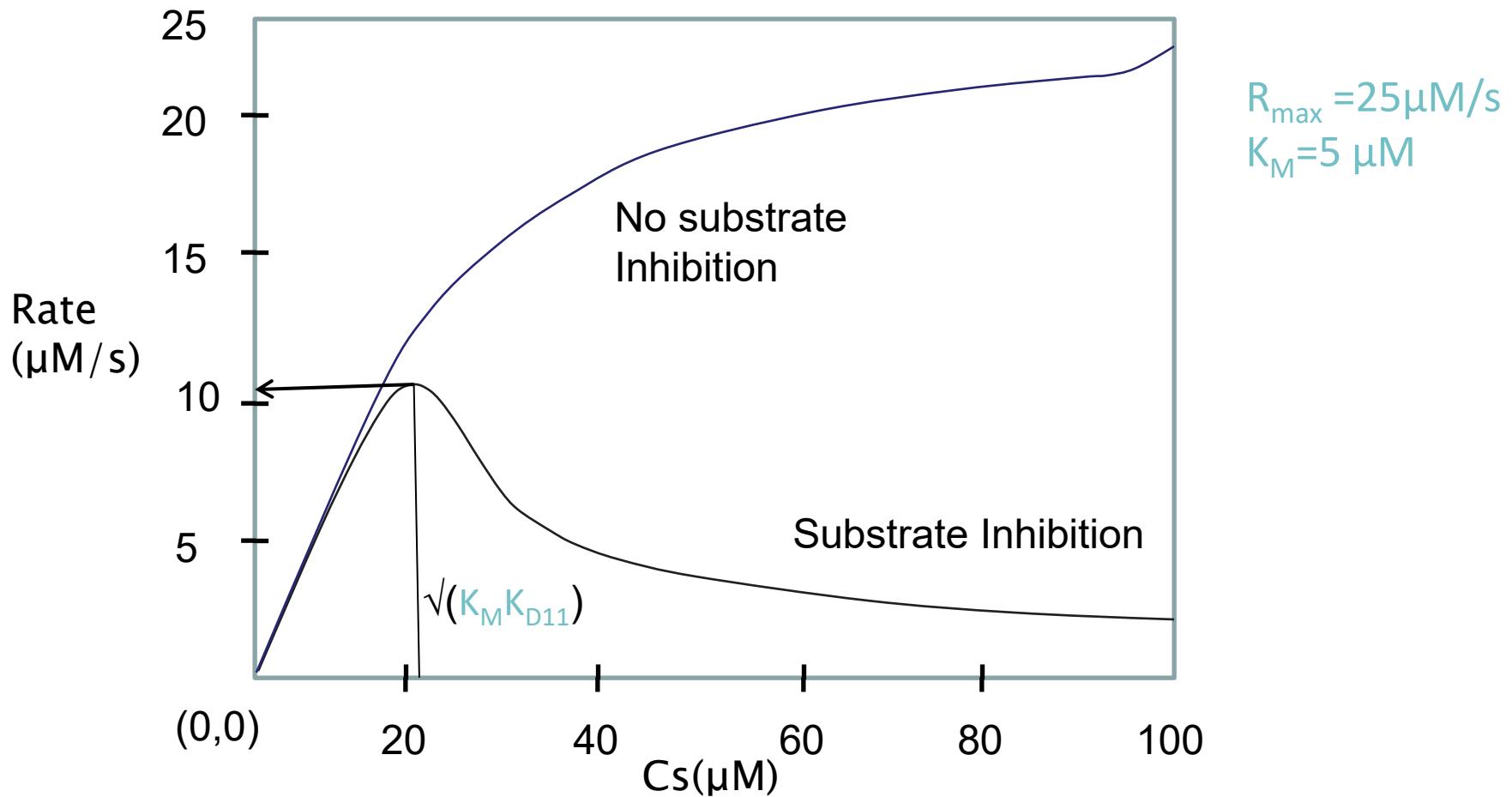
$R_{\max}$  occurs, when  $\frac{d(\text{Rate})}{dC_S} = 0$

Differentiating equation (6)

$$C_S = \sqrt{K_M K_{D11}}$$

when  $R = R_{\max}^{(s)}$

$$R_{\max}^{(s)} = \frac{K_2 C_{EO} \sqrt{K_M K_{D11}}}{2K_M + \sqrt{K_M K_{D11}}} = \frac{K_2 C_{EO} \sqrt{K_{D11}}}{2\sqrt{K_M} + \sqrt{K_{D11}}}$$



# Epistemic Qs 17: What is the most potent inhibition in Biochemical systems? Why?

## Enzyme Inhibition: Summary

Inhibitor	Binds to	Inhibition Type	$R_{max}$	Initial Rate ( $=R_{max}/K_M$ )
Inhibitor	Active Site	Competitive	Same	Decreases
Inhibitor	Non-active Site	Uncompetitive	Decreases	Same
Inhibitor	Active & Non-active Site	Non-competitive	Decreases	Decreases
Substrate	Non-active site	Substrate	Peak rate (lower than $R_{max}$ )	Same
Substrate & Inhibitor	Active site (Inhibitor) & Non-active site (Inhibitor & Substrate)	Non-competitive Substrate*	Peak rate (much lower than $R_{max}$ )	Decreases

$$*R_l = \frac{V_{max,l} C_s}{\left( K_{M,l} + C_s + \frac{C_s^2}{K_s} \right) \left( 1 + \frac{C_{xylose}}{K_{l1}} \right)},$$

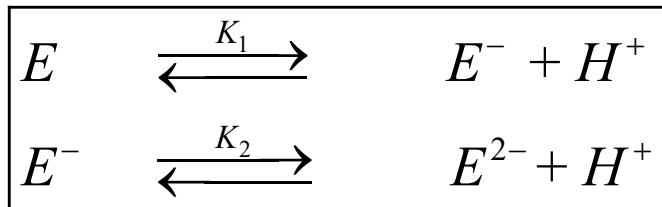
**Epistemic Qs 5.**

**Why does pH and temperature affect enzyme activity and therefore reaction kinetics?**

**Epistemic Qs 19.**

**How to calculate the optimum pH for an enzymatic reaction?**

# Effects on PH on enzyme activity (for pH sensitive enzyme)



(Both in equilibrium)

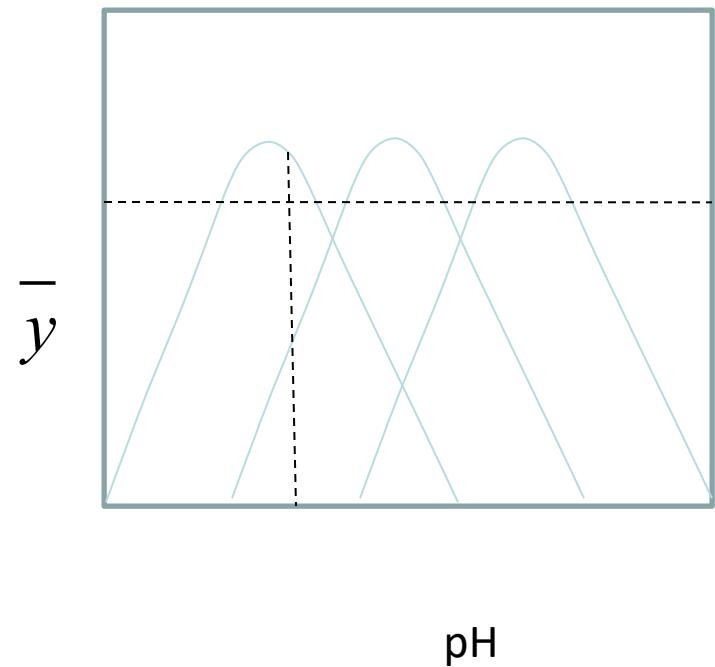
$$K_1 = \frac{[H^+][E^-]}{[E]} \quad \& \quad K_2 = \frac{[H^+][E^{2-}]}{[E^-]}$$

Constraint Eqn:

$$[E_0] = [E] + [E^-] + [E^{2-}]$$

$$= \frac{[E^-][H^+]}{K_1} + [E^-] + \frac{K_2[E^-]}{[H^+]}$$

$$\bar{y} = \frac{[E^-]}{[E_0]} = \frac{1}{\left(1 + \frac{[H^+]}{K_1} + \frac{K_2}{[H^+]}\right)}$$



$$pH = -\log_{10}[H^+]$$

$$\bar{y} = \bar{y}_{\max} \quad \text{at pH} = \frac{1}{2}(pK_1 + pK_2)$$

# Effect of temperature on enzyme activity

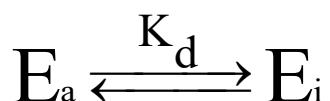
$$K = A * \text{Exp}\left(-\frac{E_a}{RT}\right)$$

$E_a$  = Activation Energy      A=frequency factor

T=temperature(at temperatures above 45°C enzymes deactivates)

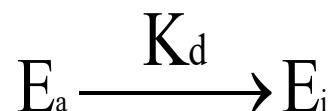
## Models of deactivation

### Reversible model



$$\frac{E_i}{E_a} = K_d = \exp\left(\frac{-\Delta G_d}{RT}\right)$$

### Irreversible model



$$E_a = [E_a]_{t=0} \exp(-k_d t)$$

$$r_d = -k_d [E_a] = \frac{d[E_a]}{dt}$$