

## Analysis of Enzymes

10/0ct/22

- Enzymes are catalysts: Homogenous and heterogenous catalysts
- Enzymes are close to heterogenous catalysts but not exactly.
- Enzymatic catalysis can be considered as a third category.
- Enzymes are proteins. Majority of proteome in a species is enzymes.
- Enzymes are highly specific.  
Metabolites are products (chemical) present in the body. It is highly likely that for every metabolite there is one enzyme.
- To name the enzyme you add an "ase" at the end of it.
- Type of reaction catalysed.
  1. Oxidoreductase - Transfer of electrons
  2. Transfase - group transfer reactions  
(-CH<sub>3</sub> grp transfer)
  3. Hydrolases - Hydrolysis reaction  
cleavage of C-C, C-N, C-O bonds
  4. Lyases - by elimination, leaving double bonds
  5. Ligases - formation of C-C, C-N, C-O bond.
  6. Isomerase - Reactions to yield isomers.

# Working of enzymes (Unimolecular reactions)

You can have - lock and key induced fit

conformation selection

(bonds in sub are weakened)

substrate →

active site

active site

enzyme

enzyme - substrate

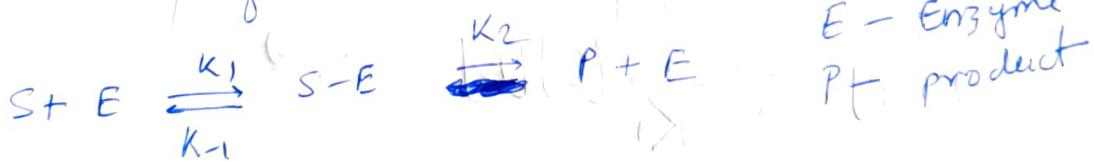
product

enzyme

- For two different substrates, similar mechanisms.

- Michaelis - Menten model

→ Observation: Enzymes do not follow the law of mass action.

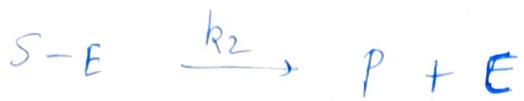
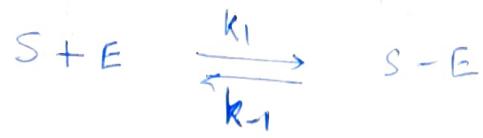


$$\frac{d[S]}{dt} = k_{-1}[S-E] - k_1[S][E]$$

$$\frac{d[E]}{dt} = (k_{-1} + k_2)[S-E] - k_1[S][E]$$

$$\frac{d[S-E]}{dt} = k_1[S][E] - (k_2 + k_{-1})[S-E]$$

$$\frac{d[P]}{dt} = k_2[S-E]$$



$[S-E]$  can't be determined experimentally.

so we have to eliminate it

Ultimately I need  $\sigma = f([S] \text{ or } [P])$

→ Equilibrium approximation

• Eqm is reached instantaneously



$$k_1 [S][E] = k_{-1} [S-E]$$

$$(r_f = r_b)$$

(rate of forward rxn  
and backward rxn is  
assumed to be equal  
right from  $t=0$ )

$$\Rightarrow [S-E] = \frac{k_1 [S][E]}{k_{-1}}$$

$$[S-E] = \frac{1}{K_1} [S][E] \quad \text{--- (2)}$$



$$V = k_2 [S-E] \quad (\text{velocity of the rxn})$$

$$V = \frac{k_2 [S][E]}{K_1} \quad (\text{rate of rxn}) \quad \text{--- (3)}$$

- How to get rid of E?

Enzyme balance:

$$[E](t) + [E-s](t) = [E]_0 \quad (t=0)$$

$\begin{matrix} \text{unreacted} & \text{reacted} \\ \text{enzyme @ } t & \text{enzyme @ } t \end{matrix} = \begin{matrix} \text{Initial enzyme} \\ \text{unreacted enzyme} \\ (@ t=0) \end{matrix}$

$$\begin{aligned} [E]_0 &= [E] + [E-s] \\ &= [E]_0 + \frac{[S][E]}{K_1} \end{aligned} \quad \begin{matrix} [E]_0 - \text{Initial} \\ \text{unreacted enzyme} \end{matrix}$$

$$[E] = \frac{K_1 [E]_0}{K_1 + [S]} \quad (4)$$

⇒ From ③ and ④

$$V = \frac{k_2 [E]_0 [S]}{K_1 + [S]}$$

$$V = \frac{V_{\max} [S]}{K_1 + [S]}$$

$$V_{\max} = k_2 [E]_0$$

→ Quasi-steady state approximation.

- $[S-E]$  will be consumed the instant it is formed.

All intermediates are such that they'll get consumed the moment they are formed.

⇒ rate of formation  $[S-E] =$  rate of consumption  $[S-E]$

$$k_1[S][E] = k_1[S-E] + k_2[S-E]$$

$$\Rightarrow [S-E] = \frac{k_1}{k_1 + k_2} [S][E]$$

$$= \frac{1}{K_M} [S][E]$$

$$K_M = \frac{k_1 + k_2}{k_1} \quad \begin{array}{l} \text{(eqm of all forward)} \\ \text{(eqm of all backward)} \end{array}$$

$$\vartheta = \frac{k_2}{K_M} [S][E]$$

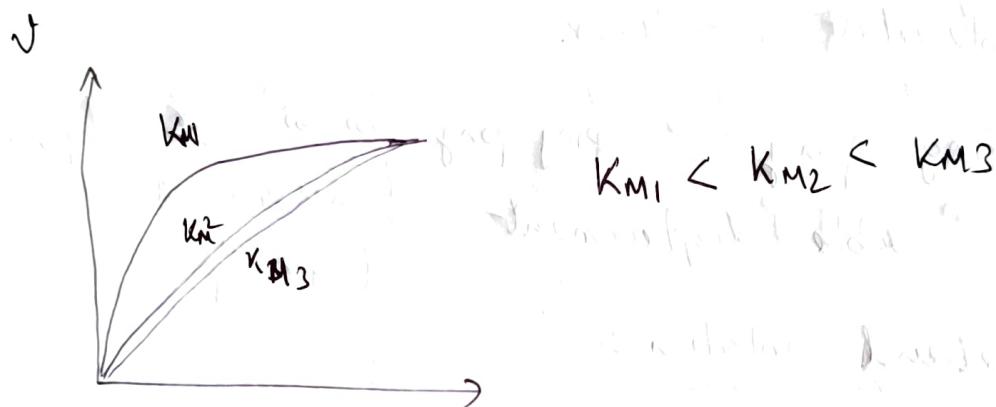
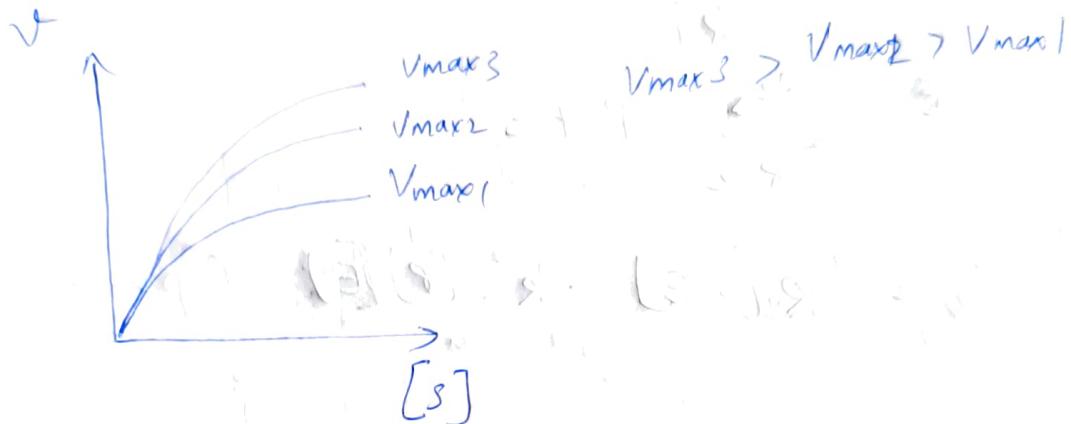
Again enzyme balance:

$$\begin{aligned}[E]_0 &= [E] + [E-S] \\ &= [E] + \frac{1}{K_M} [E][S]\end{aligned}$$

$$E = \frac{K_M [E]_0}{K_M + [S]}$$

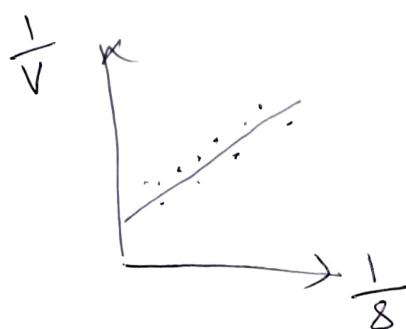
$$\Rightarrow v = \frac{V_{max} [S]}{K_m + [S]}$$

$$V_{max} = k_2 [E]_0$$



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

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

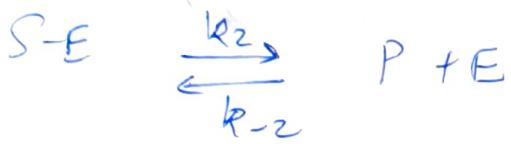
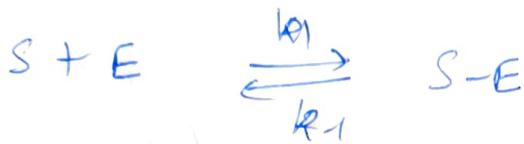


Some gyaan on curve fitting. MATLAB use Karlo laro <3.

Lineweaver - Burk method

Cogish - Boudon

Reversible reaction (unimolecular)



$$\vartheta = k_2[S-E] - k_{-2}[P][E] \quad (\text{photo})$$

Bimolecular reactions.

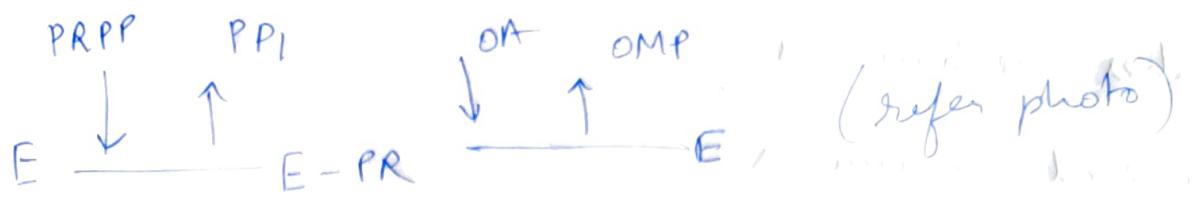
- Ping-pong  $\rightleftharpoons$  ping-pong bi-bi  $\rightleftharpoons$  (photo)  
double displacement

Cleland notation :

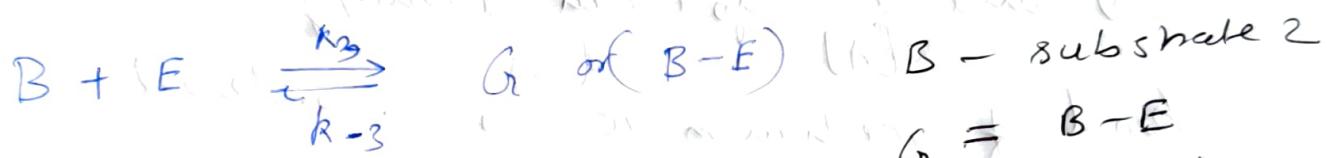
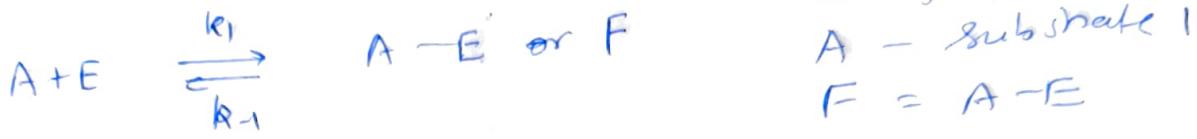
→ Substrates are designated A, B, C and D  
in the order they bind to the enzyme.

→ Products - P, Q, R and S, in the order  
they leave enzyme.

→ Stable enzymes : E, F, G, with E being  
the free enzyme.



for example:



$$[E]_0 = [E] + [F] + [G]$$

In quasi-steady state approx, (Sir ne kya class me dikhaaya)

$$V = \frac{k_1 k_2 k_3 k_4 [E]_0 [A][B]}{---}$$

(see photo too)

## Other mechanisms:

- Ordered mechanism
- Random kinetic mechanism.
- Depending on the nature of plots between  $\frac{1}{[A]}$  and  $\frac{1}{V_r}$  you can identify what kind of mechanism it is - ordered or ping pong or random-kinetic.
- In ping-pong models the curves are parallel whereas in ordered they come off

# Enzyme Inhibition

17/10/22

- Inhibition reduces the speed of the reaction.  
in presence of inhibitor the rate of rxn is reduced.
- Inhibitor should also have geometric and electrostatic fit.
- Inhibitor is also a substrate.
- Why would the enzyme react with inhibitor? rather than the substrate.



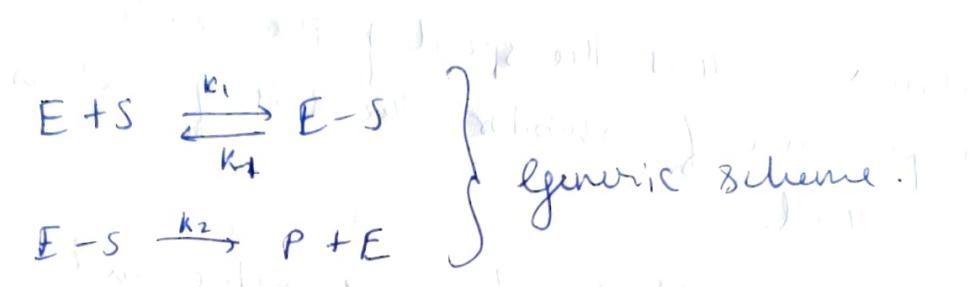
$$\rightarrow \Delta G_1 = G_{\text{enzyme} - \text{substrate}} - G_{\text{substrate}} - G_{\text{enzyme}}$$
$$\Delta G_2 = G_{\text{enzyme} - \text{inhibitor}} - G_{\text{inhibitor}} - G_{\text{enzyme}}$$

The enzyme combines with inhibitor because  $\Delta G_2$  of this reaction is more negative than enzyme - substrate reaction.

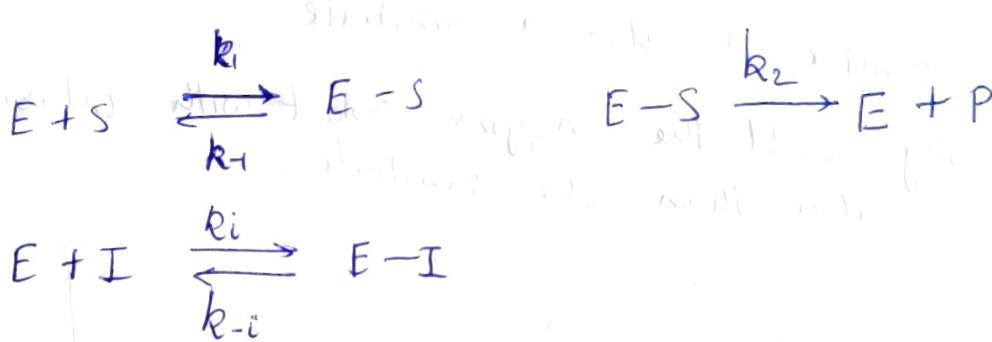
$$|\Delta G_2| > |\Delta G_1|$$

- There is some probability distribution (maxwell energy distribution)

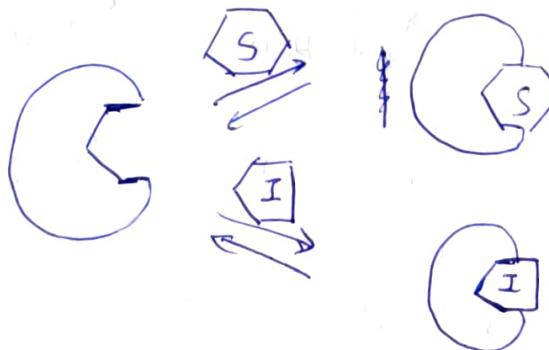
# I. Enzyme Inhibition : Competitive inhibition



In presence of inhibitor



→ Inhibitors basically blocks the spot where substrate would have come.



$$v = k_2 [E - s] \quad \text{--- ①}$$

Quasi - SS

$$k_1 [E][s] = k_{-1} [E - s] + k_2 [E - s]$$

$$[E - s] = \frac{1}{K_m} [E][s]$$

$$k_i [E][I] = k_{-i} [E - I]$$

$$v = \frac{k_2}{K_m} [E][s]$$

Enzyme deactivation -

$$[E]_0 = [E] + [E - I] + [E - s]$$

$$[E]_0 = [E] + \frac{1}{K_m} [E][s] + \frac{1}{K_I} [E][I]$$

$$v = \frac{k_2}{K_m} \left[ \frac{[E]_0 [s]}{1 + \frac{[s]}{K_m}} + \frac{[I]}{K_I} \right]$$

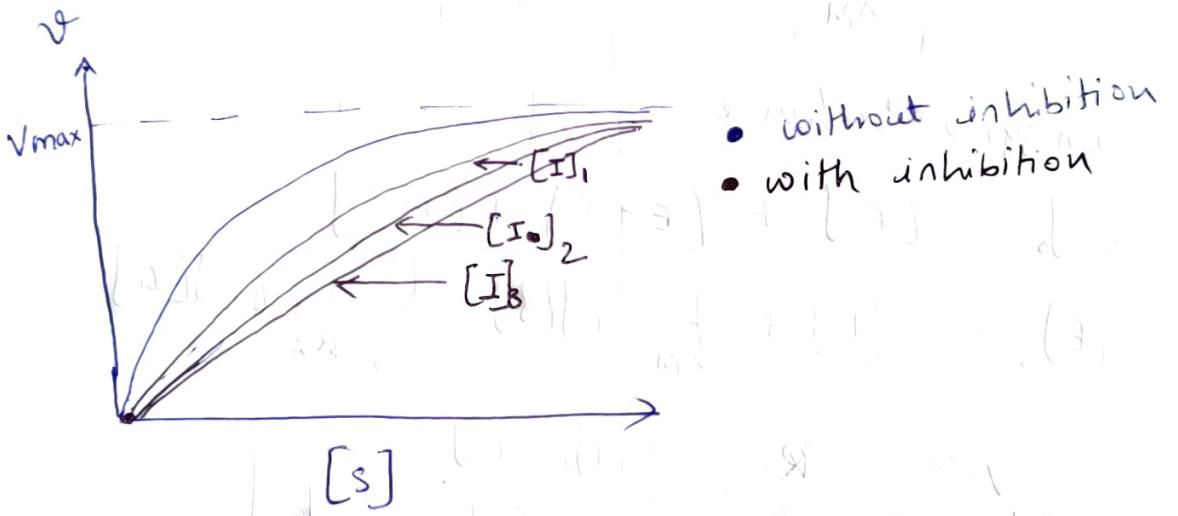
$$v = \frac{k_2 [E]_0 [s]}{K_m + \frac{K_m}{K_e} [I] + [s]}$$

$$v = \frac{V_{max} [s]}{K_m \left( 1 + \frac{[I]}{K_I} \right) + [s]}$$

$$v = \frac{V_{max} [s]}{K_m \left( 1 + \frac{[I]}{K_i} \right) + [s]}$$

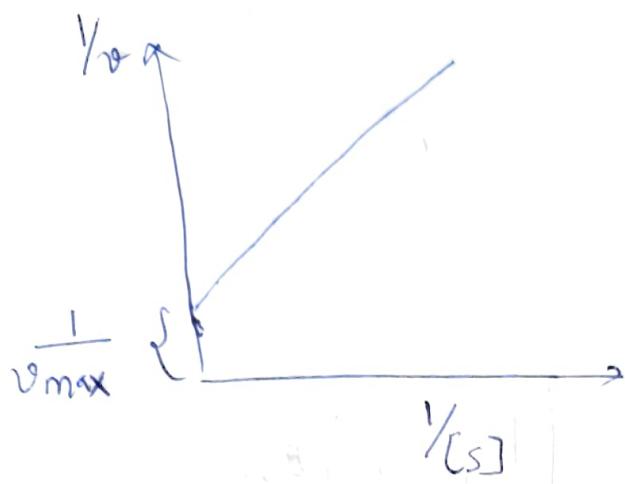
$$v = \frac{V_{max} [s]}{K_m + [s]} \quad [I]_0 = \text{const.}$$

$$\alpha = 1 + \frac{[I]}{K_i}$$

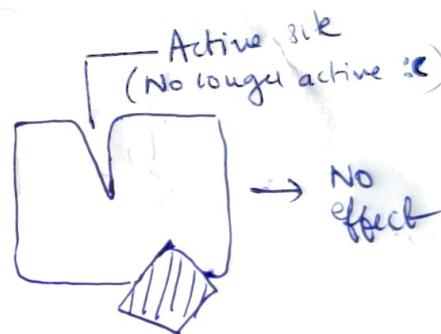
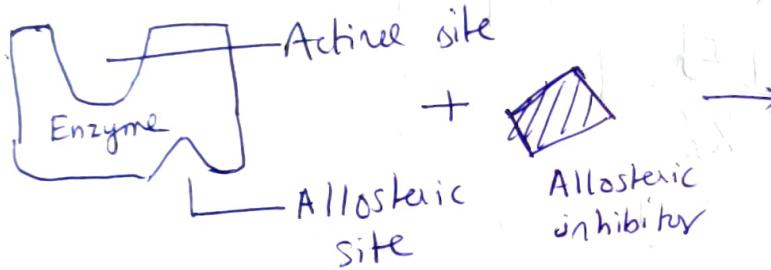


- with or without inhibition  $V_{max}$  remains constant.
- $[I]_1 < [I]_2 < [I]_3$  (with increase in inhibitor concentration rate of reaction decreases)
- With inc in  $K_i$  rate of reaction increases.

$$\frac{1}{v} = \left( \frac{\alpha K_m}{V_{max}} \right) \frac{1}{[S]} + \frac{1}{V_{max}}$$

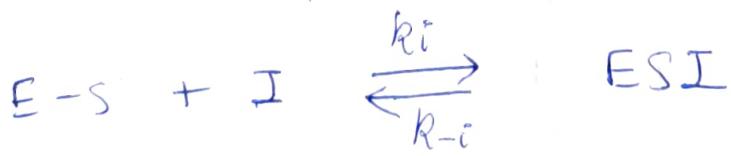
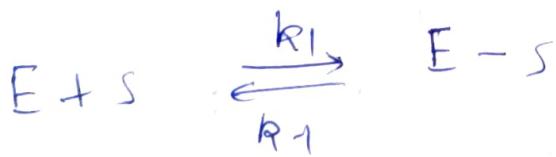


## 2. Allostery (uncompetitive inhibition)



→ This inhibition can be activating or deactivating.

(photo)



$$V = k_2 [E - S]$$

$$[E - S] =$$

$$[E - S - I] =$$

$$[E]_0$$

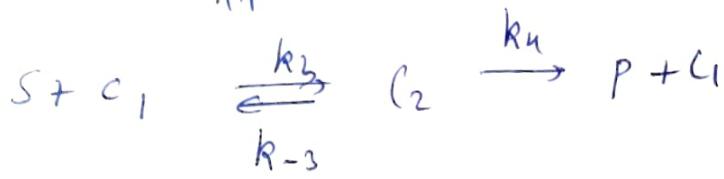
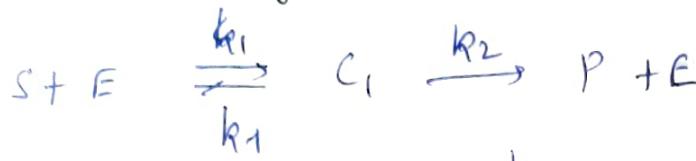
$$V = \frac{v_{\max}}{\frac{\alpha'}{\frac{K_m}{\alpha'} + [S]}} [S]$$

$$V = \frac{k_2 [E]_0}{1 + \frac{[I]}{K_I} + [S]}$$

$$\alpha' = 1 + \frac{[I]}{K_I}$$

Mixed Inhibition  
when  $K_I = K_I'$   $\rightarrow$  Non-competitive.

Cooperativity in enzyme catalysis.



$$v = \frac{(k_2 k_2 + k_4 [S])([E]_0 [S])}{K_1 K_2 + K_2 [S] + [S]^2}$$

Quasi Steady State

$$[E]_0 = [E] + [C_1] + [C_2]$$

case 1: Binding sites act independently and identically.

$$k_1 = 2 k_3 = 2 K +$$

$$2 K_{-1} = K_{-3} = 2 K -$$

$$2 k_2 = k_4$$

$$v = 2 \frac{k_2 [E]_0 [S]}{K + [S]}$$

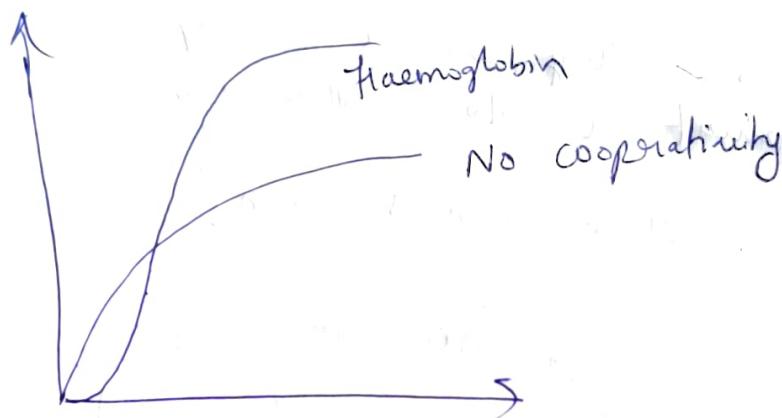
$$K = \frac{K-1 + k_2}{K+}$$

case 2: The binding at the second site is faster when the first site is occupied

$$v = \frac{k_4 [E]_0 [S]^2}{K_m^2 + [S]^2}$$

- Binding of oxygen to haemoglobin and myoglobin.

- Myoglobin and haemoglobin
- Haemoglobin has 4 units and thus cooperativity occurs.



## Analysis of Metabolism

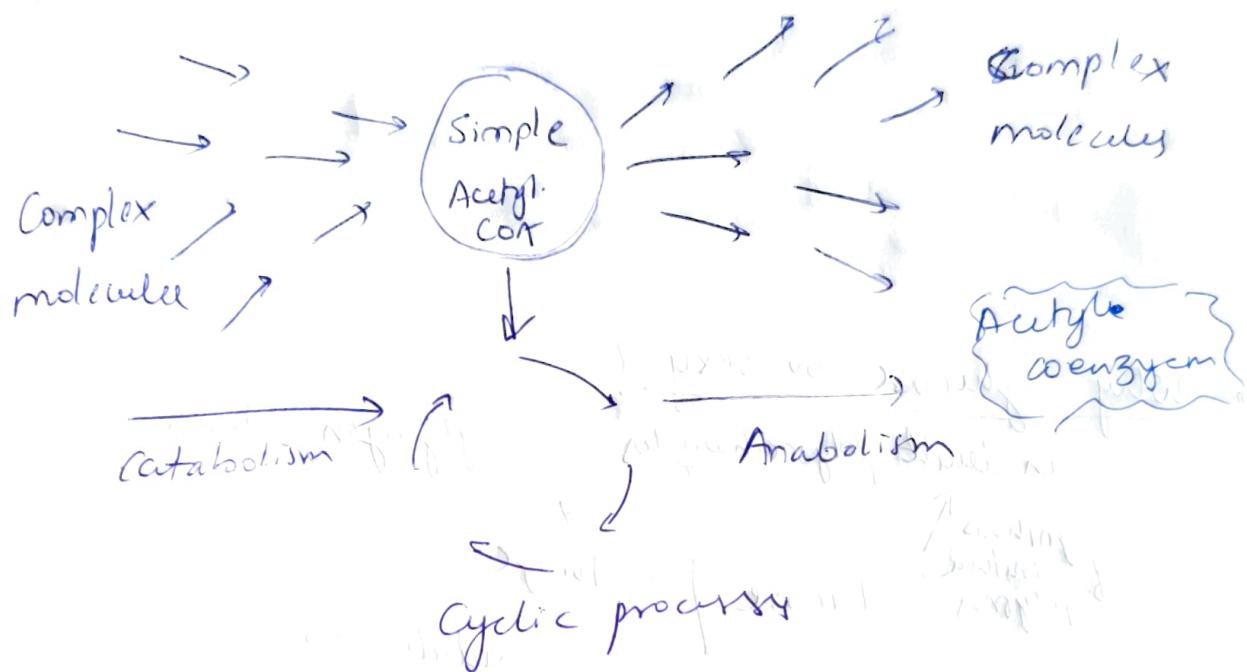
- Metabolism will help you understand how does your body process a molecule. For example a metabolism to "process" sugar molecule.
- You eat food → food gets converted to biomass.
- Heterotrophs - they consume one type of biomass and convert it into another type of biomass

Autotrophs - Make biomass, from?

- ~~Simple~~ → complex → Anabolism ("Processing" sugar)  
complex → simple → Catabolism.
- Anabolism: formation of complex molecules from simple molecules i.e. the size is growing, its called anabolism.
- catabolism: breaking down complex molecules to simpler example: sugar to carbon dioxide
- During there is formation / consumption of energy.

→ Catabolism is converging, anabolism is diverging  
converges to a simple molecule Acetate

which is Acetyl CoA



i) Generation and consumption of energy  
Phosphorylation. (glucose can accept phosphate group)



- Conversion of ATP to ADP releases energy because  $E(ATP) > E(ADP)$  as ATP has a large concentration of charged groups as compared to ADP
- Glucose molecule accepts phosphate group and converts  $ATP \rightarrow ADP$
- Metabolite - glucose

## 2) Redox (Species can accept or release H)

NAD - Nicotinamide Adenosine d



Metabolite -

why glucose so sexy?

can deint polysaccharides

Synthesis  
of  
structural  
polymers

Glucose

Glycogen, starch,  
sucrose

Storage

Oxidation via  
glycolysis

Pyruvate

formation of ribose-5-phosphate  
for RNA formation

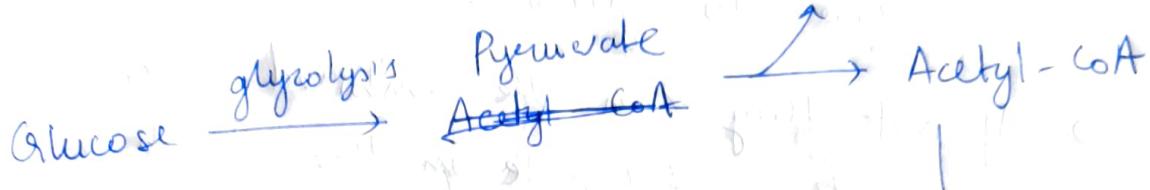
This branch is  
too sexy. This is where  
most glucose goes to.

→ Morning assembly fainting example.

## Glycolysis : Krebs cycle : transfer

→ Series of reactions to break down glucose into simple molecules called pyruvate.

→ You accept



→ Krebs cycle : Citric acid cycle  
: TCA cycle

→ Glycolysis.



→ Krebs cycle ke output  
is  $CO_2$ ,  $H_2O$  and  
lot of different  
metabolites.

All this takes place in mitochondria.

Electron  
proton  
transfer

- ATP me use store energy  
useful energy and it is given in  
terms of ATP.
- All kinds of sugars in one way or  
other to glucose.
- If not they can be combined with  
a ~~useless~~ metabolite in the Krebs  
cycle and then form an intermediate  
which will release energy.

Lack of glucose and inefficiency of glucose processing.

- If you run out of glucose your body uses fat to extract energy.
- The mechanisms we learnt do not apply for fats. So how do we extract energy?
- When you start using fats for energy consumption your mechanism changes from glucose-based to fat based. pH of blood changes. Ketones are formed.
- Pancreas release insulin. There are vesicles in blood which are formed because of insulin. These vesicles attach to glucose and take glucose to the mitochondria. and take glucose to the mitochondria. so if the pancreas don't work well, the insulin level will decrease and the vesicles won't be formed and glucose won't reach mitochondria.
- Glucose will then be present in blood stream and body will start using fats for energy metabolism. pH increases. More ketone bodies.

## Pyruvate utilisation

### Alcohol dehydrogenase

Ethanol  $\rightarrow$  carboxylic acid  
which are charged. So when this goes ~~to~~ to the brain then it interferes.

Conversion of ethanol to carboxylic acid takes place in liver.

- Alcohol manufacturing leads to formation of congeners. Congeners are the product except (organic compounds) except ethanol formed during alcohol manufacturing.
- Beer and wine are not distilled.
- Distilled drinks have 40% volume ethanol.
- Aasavan

## ANALYSIS OF ENZYMES

(10 OCT 2022)

Michaelis-Menten kinetics: Reversible reaction scheme

$$S + E \xrightleftharpoons[k_{-1}]{k_1} S-E \xrightleftharpoons[k_{-2}]{k_2} P + E \quad (12)$$

Equilibrium approximation

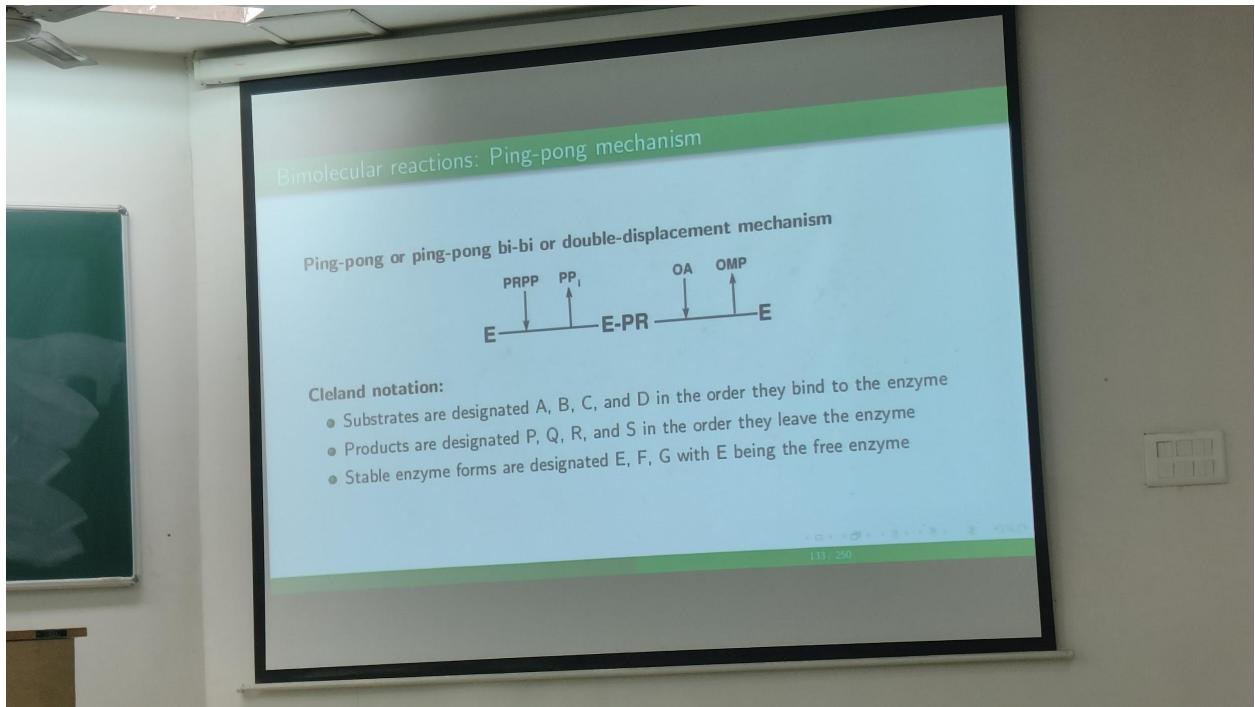
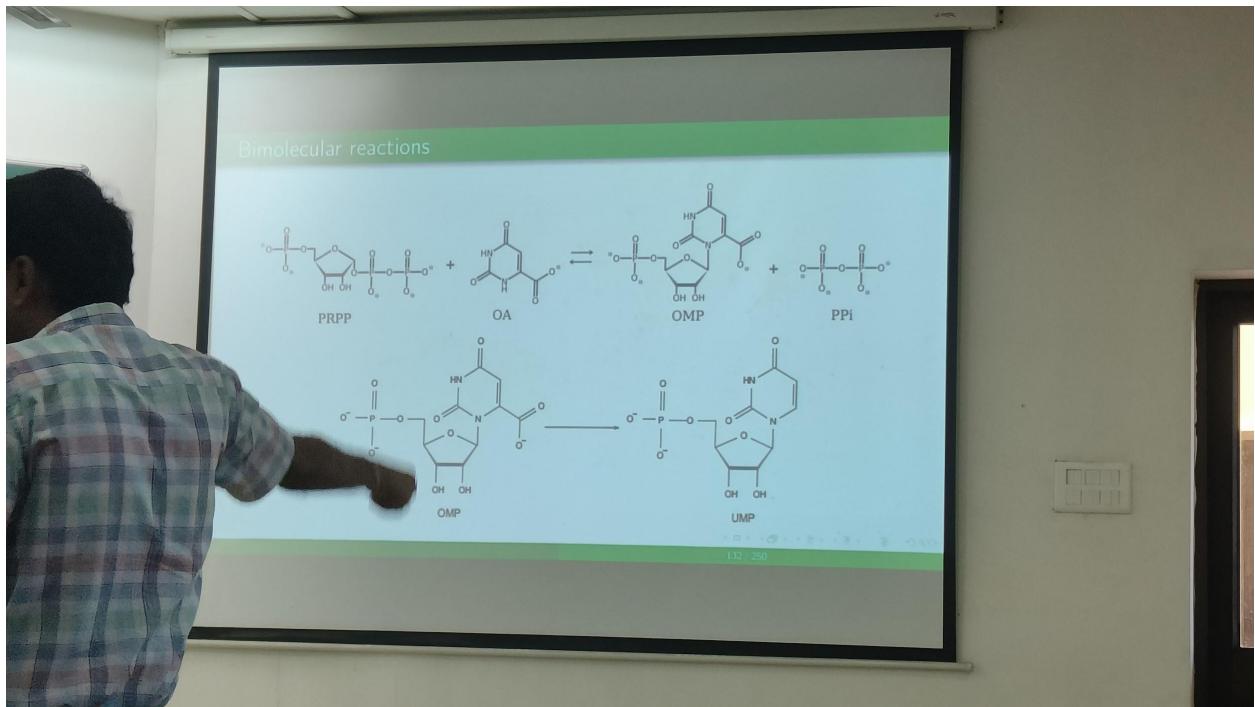
$$V = [E]_0 \frac{k_1 k_2 [S] - k_{-1} k_{-2} [P]}{k_1 [S] + k_{-1}} \quad (13)$$

Quasi-steady-state approximation

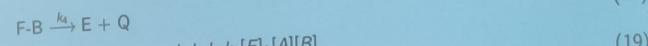
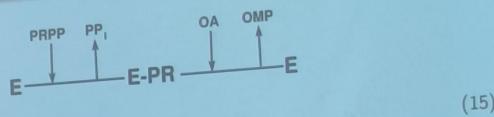
$$V = [E]_0 \frac{k_1 k_2 [S] - k_{-1} k_{-2} [P]}{k_1 [S] + k_{-2} [P] + k_{-1} + k_2} \quad (14)$$

Michaelis-Menten kinetics

Equilibrium, irreversible	Quasi-steady-state, irreversible
$V = \frac{V_{max}[S]}{K_1 + [S]}$	$V = \frac{V_{max}[S]}{K_M + [S]}$
Equilibrium, reversible	Quasi-steady-state, reversible
$V = [E]_0 \frac{k_1 k_2 [S] - k_{-1} k_{-2} [P]}{k_1 [S] + k_{-1}}$	$V = [E]_0 \frac{k_1 k_2 [S] - k_{-1} k_{-2} [P]}{k_1 [S] + k_{-2} [P] + k_{-1} + k_2}$

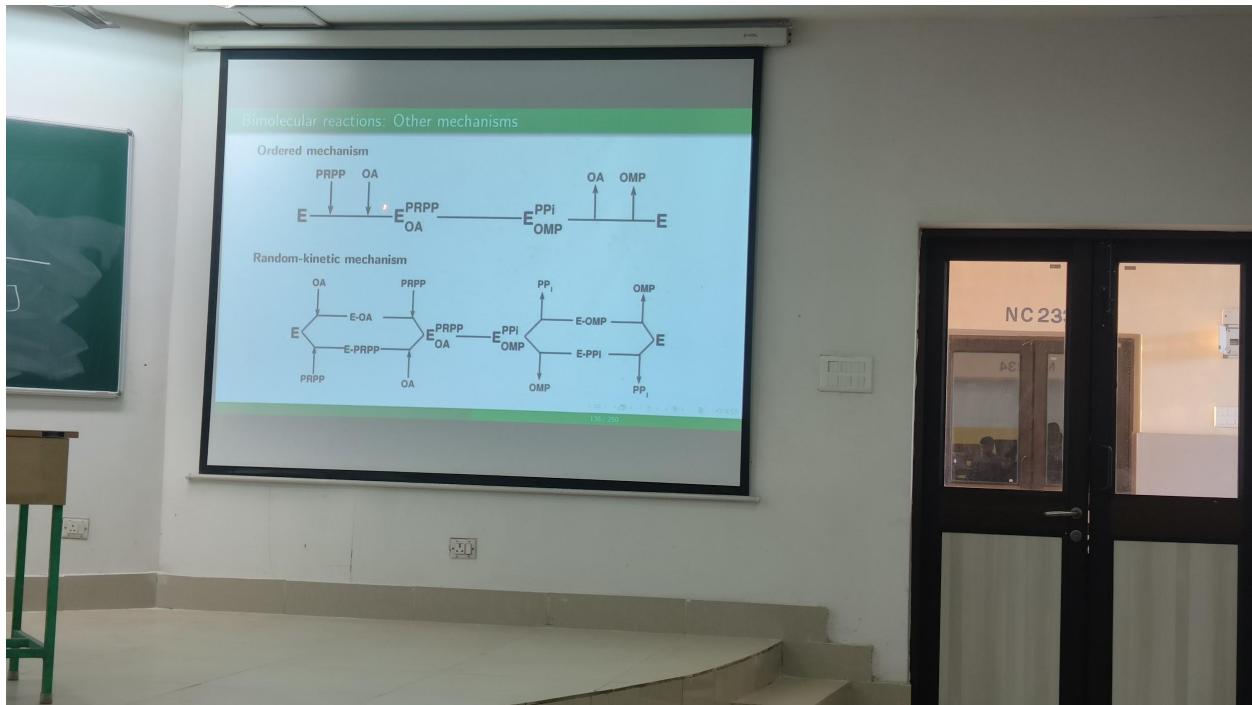


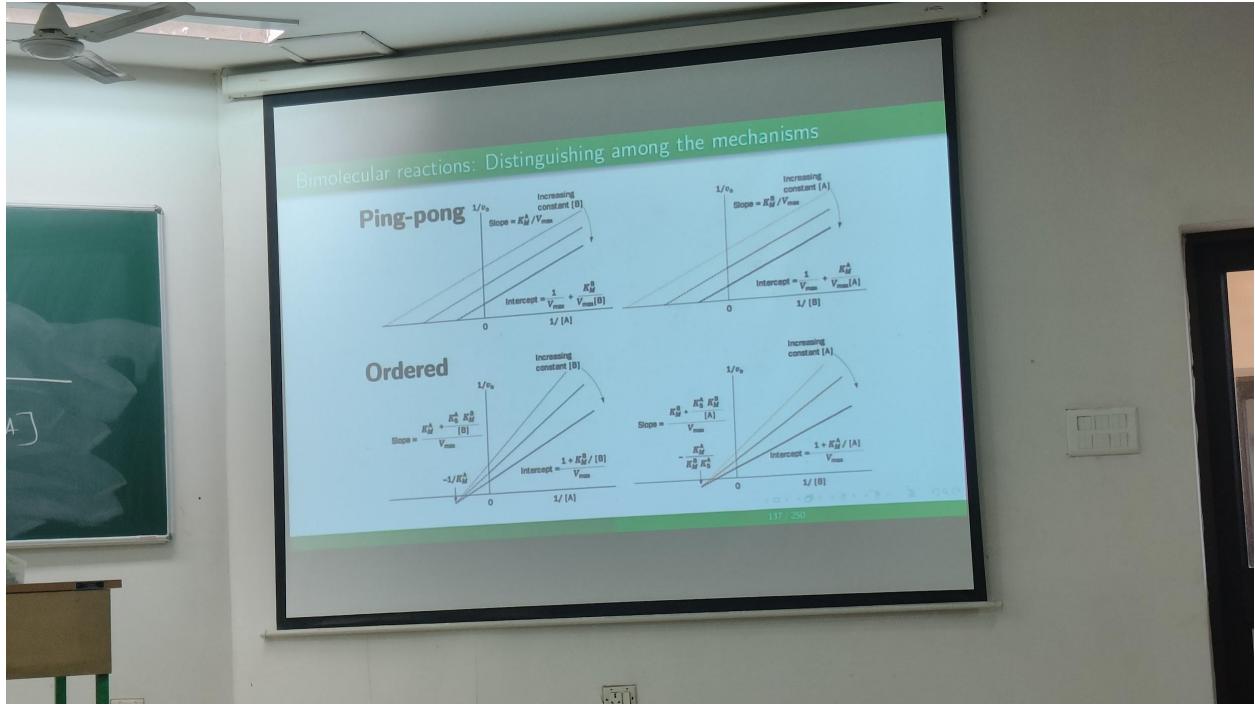
### Bimolecular reactions: Ping-pong mechanism



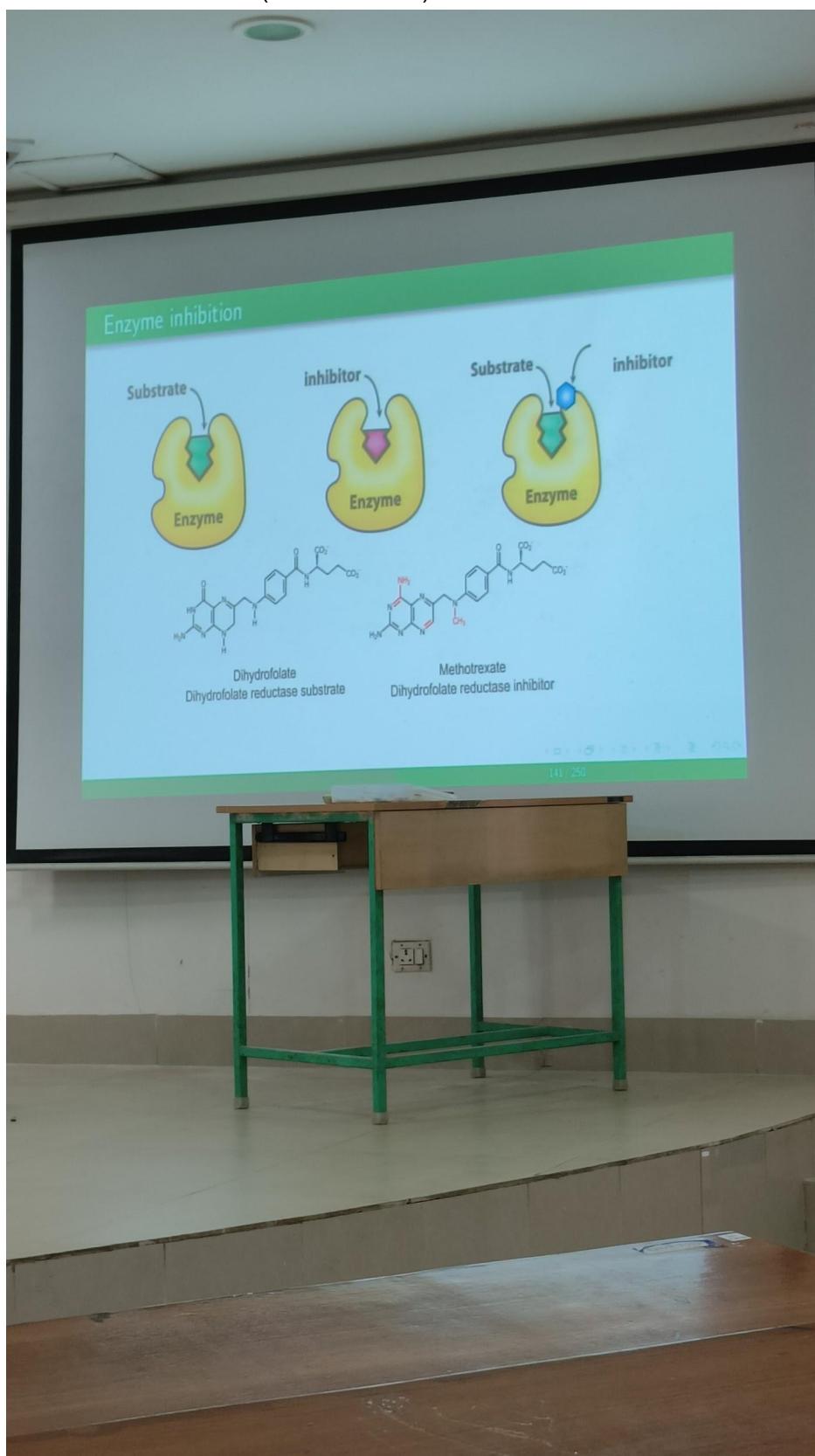
$$V = \frac{k_1 k_2 k_3 k_4 [E]_0 [A][B]}{k_1 k_2 [A] + (k_{-1} + k_2) k_3 k_4 [B] + k_1 k_3 (k_2 + k_4) [A][B]}$$

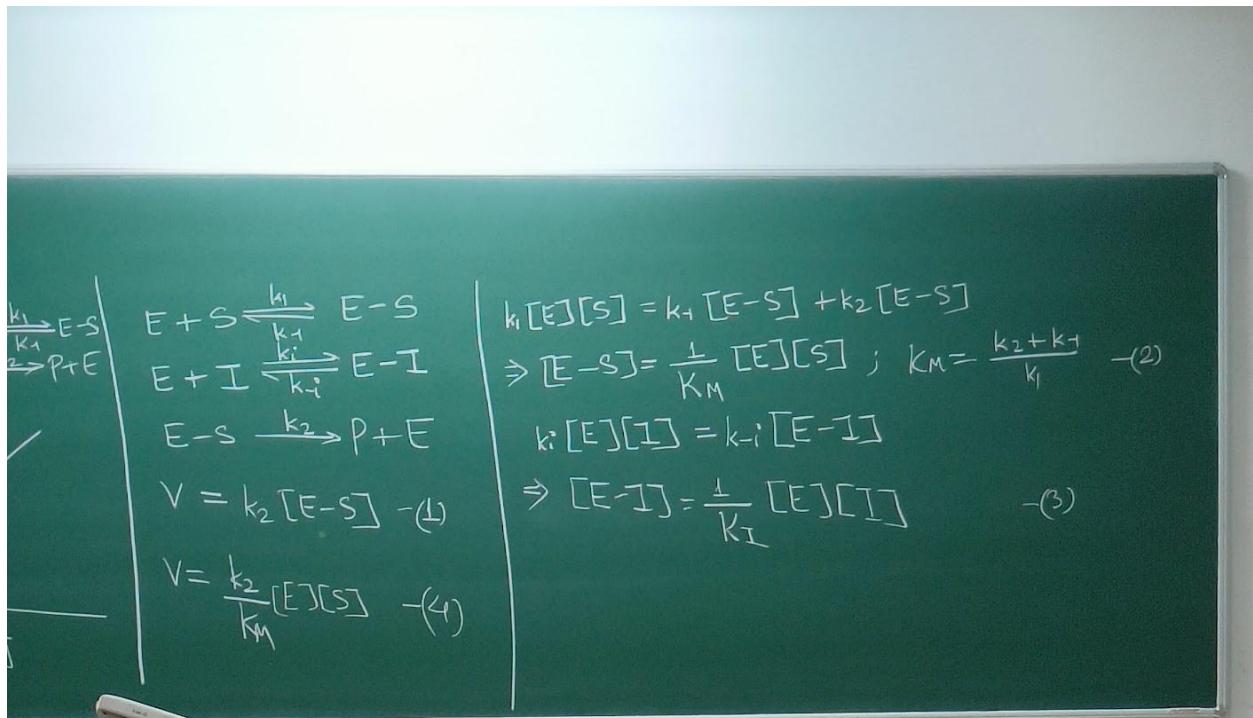
134 / 259

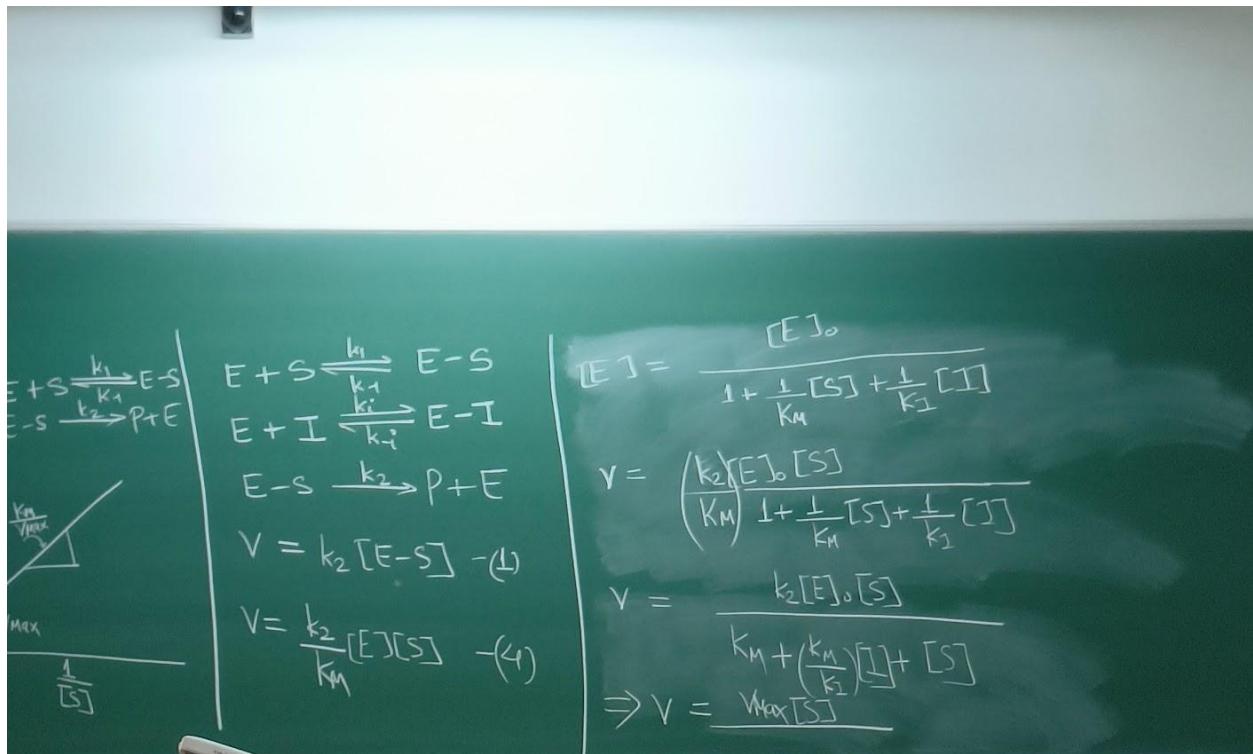


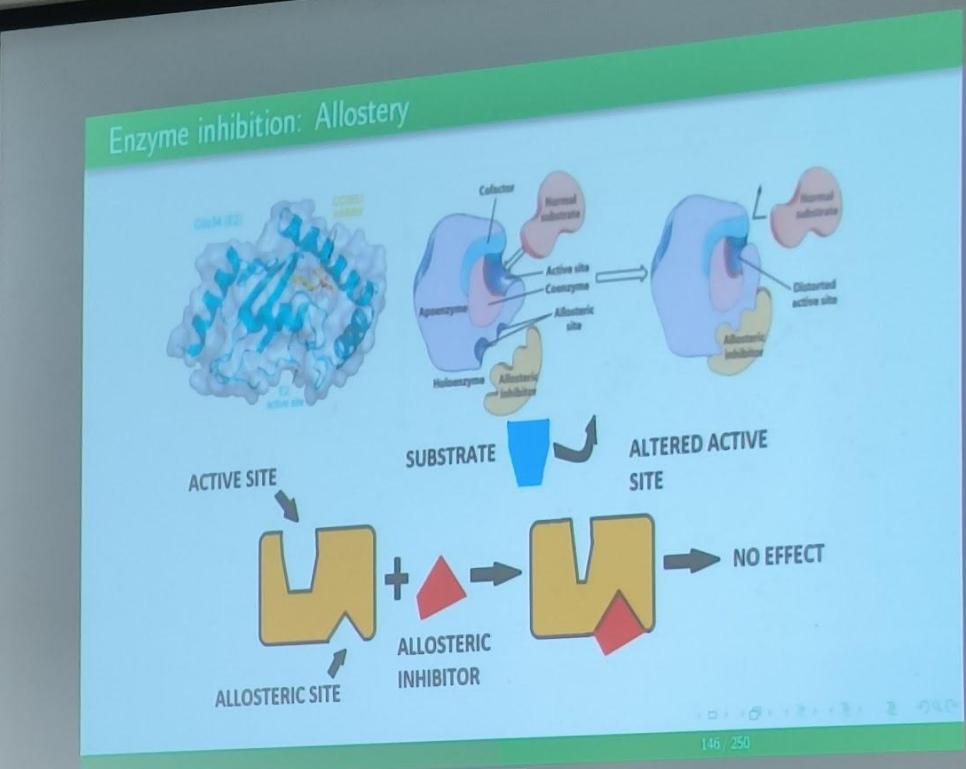


## ENZYME INHIBITION (17 OCT 2022)



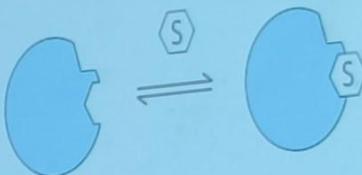
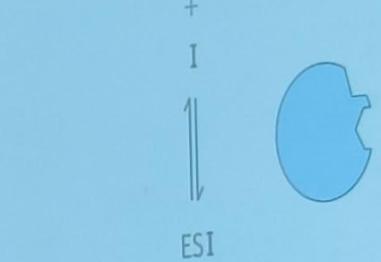
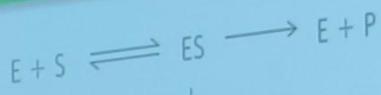






146 / 250

## Enzyme inhibition: Uncompetitive inhibition



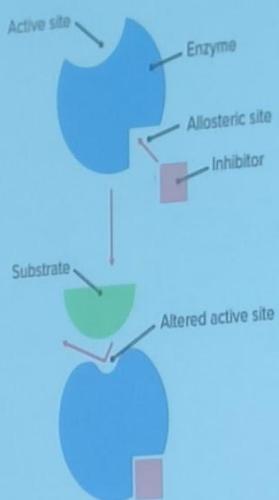
ESI

I

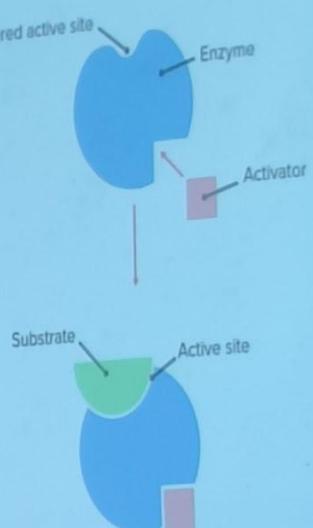


## Enzyme inhibition: Allostery

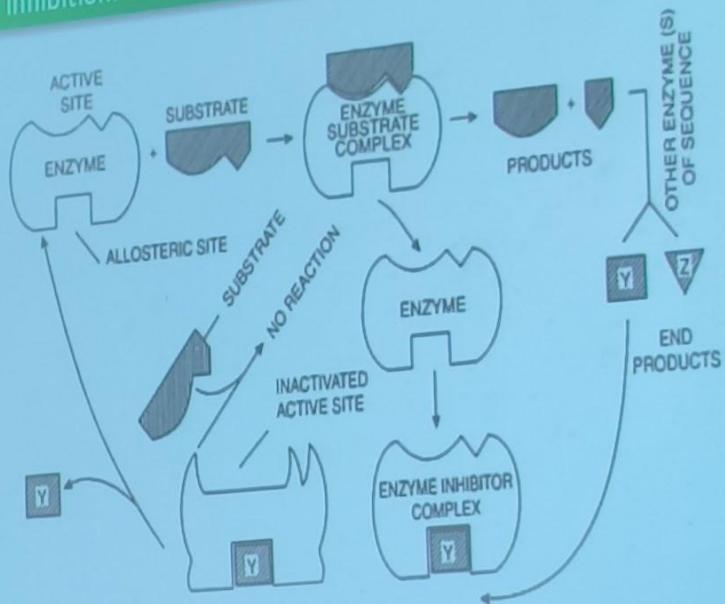
### Allosteric Inhibition



### Allosteric activation



## Enzyme inhibition: Allostery



## Enzyme inhibition: Uncompetitive inhibition

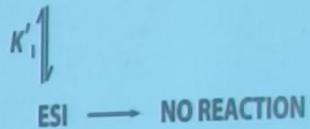
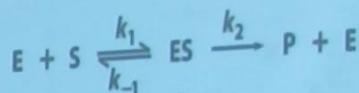
$$V = \frac{\frac{k_2[E]_0[S]}{1+\frac{[I]}{K'_I}}}{\frac{K_M}{1+\frac{[I]}{K'_I}} + [S]}$$

(22)

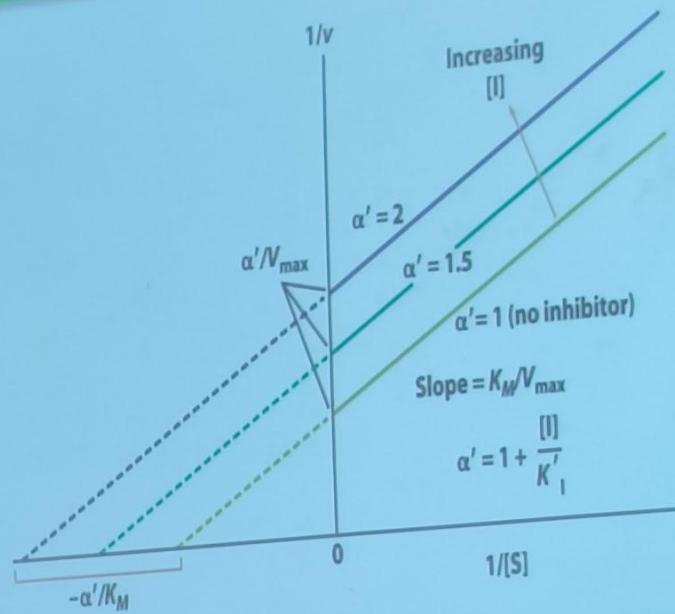
$$V = \frac{\frac{V_{max}[S]}{\alpha' + [S]}}{\frac{K_M}{\alpha'} + [S]}$$

(23)

$$\alpha' = 1 + \frac{[I]}{K'_I}$$



## Enzyme inhibition: Uncompetitive inhibition



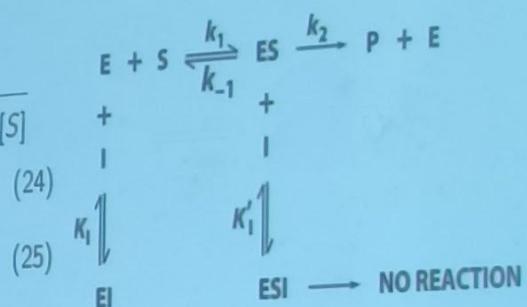
## Enzyme inhibition: Mixed inhibition

$$V = \frac{k_2 [E]_0 [S]}{K_M \left(1 + \frac{[I]}{K_I}\right) + \left(1 + \frac{[I]}{K'_I}\right) [S]} \quad (24)$$

$$V = \frac{V_{max} [S]}{\alpha K_M + \alpha' [S]} \quad (25)$$

$$\alpha = 1 + \frac{[I]}{K_I}$$

$$\alpha' = 1 + \frac{[I]}{K'_I}$$



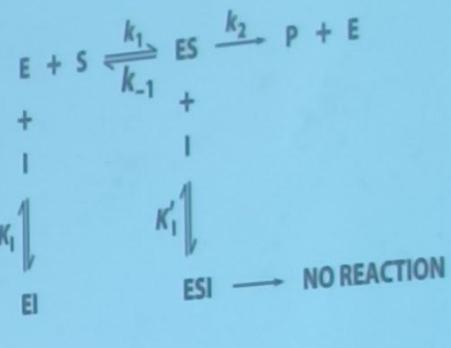
## Enzyme inhibition: Mixed inhibition

$$V = \frac{k_2[E]_0[S]}{K_M \left(1 + \frac{[I]}{K_I}\right) + \left(1 + \frac{[I]}{K'_I}\right)[S]} \quad (24)$$

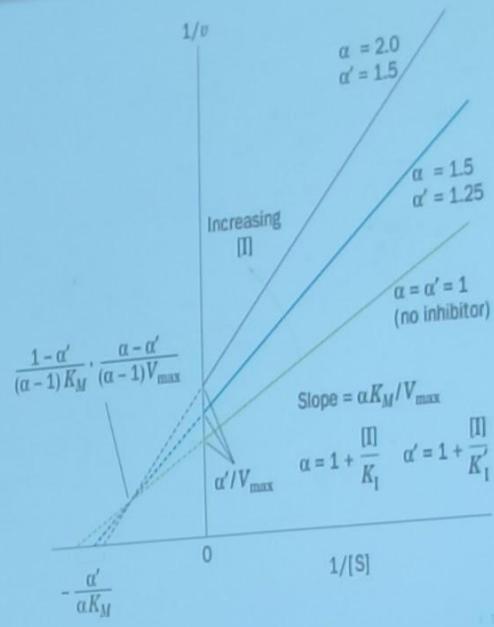
$$V = \frac{V_{max}[S]}{\alpha K_M + \alpha'[S]} \quad (25)$$

$$\alpha = 1 + \frac{[I]}{K_I}$$

$$\alpha' = 1 + \frac{[I]}{K'_I}$$



## Enzyme inhibition: Mixed inhibition



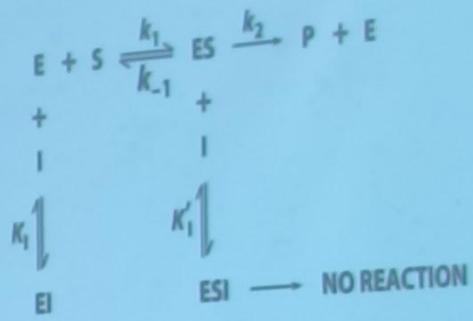
155 / 250

## Enzyme inhibition: Non-competitive inhibition

$$V = \frac{k_2 [E]_0 [S]}{K_M \left(1 + \frac{[I]}{K_I}\right) + \left(1 + \frac{[I]}{K'_I}\right) [S]} \quad (26)$$

$$\alpha = 1 + \frac{[I]}{K_I} = \alpha' = 1 + \frac{[I]}{K'_I}$$

$$V = \frac{\frac{V_{max}}{\alpha} [S]}{K_M + [S]} \quad (27)$$



## Enzyme inhibition: Comparison

(28)

$$V = \frac{V_{max}^{app}[S]}{K_M^{app} + [S]}$$

Type of Inhibition	$V_{max}^{app}$	$K_M^{app}$
None	$V_{max}$	$K_M$
Competitive	$V_{max}$	$\alpha K_M$
Uncompetitive	$V_{max}/\alpha'$	$K_M/\alpha'$
Mixed	$V_{max}/\alpha'$	$\alpha K_M/\alpha'$

$$\alpha = 1 + \frac{[I]}{K_I}$$

$$\alpha' = 1 + \frac{[I]}{K'_I}$$

## Cooperativity in enzyme catalysis



$$V = \frac{(k_2 K_2 + k_4 [S]) [E]_0 [S]}{K_1 K_2 + K_2 [S] + [S]^2} \quad (31)$$

where

$$K_1 = \frac{k_{-1} + k_2}{k_1} \text{ and } K_2 = \frac{k_{-3} + k_4}{k_3}$$

## Cooperativity in enzyme catalysis

Case 1: Binding sites act independently and identically

$$k_1 = 2k_3 = 2k_+ ; 2k_{-1} = k_{-3} = 2k_- ; 2k_2 = k_4 \quad (32)$$

$$V = 2 \frac{k_2 [E]_0 [S]}{K + [S]} \quad (33)$$

where

$$K = \frac{k_- + k_2}{k_+} \quad (34)$$



## Cooperativity in enzyme catalysis

Case 2: The binding at the second site is faster when the first site is occupied

(35)

$$V = \frac{k_4 [E]_0 [S]^2}{K_M^2 + [S]^2}$$

where

(36)

$$K_M^2 = K_1 K_2$$

Hill equation

(37)

$$V = \frac{V_{max} [S]^n}{K_M^n + [S]^n}$$

where

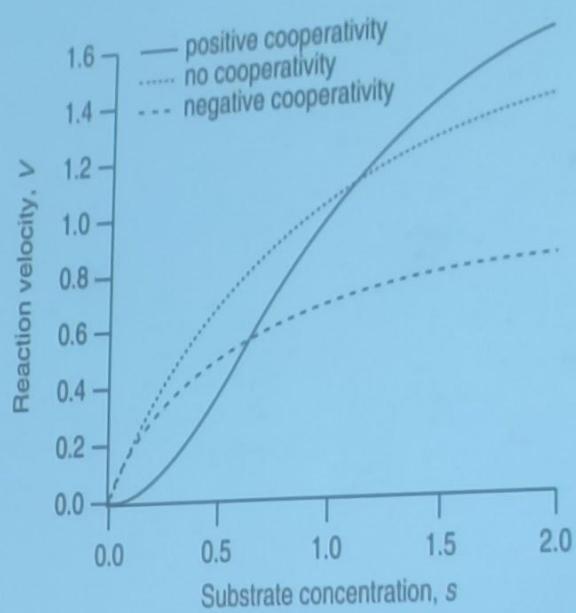
(38)

$$K_M^n = K_1 K_2 \dots K_n$$

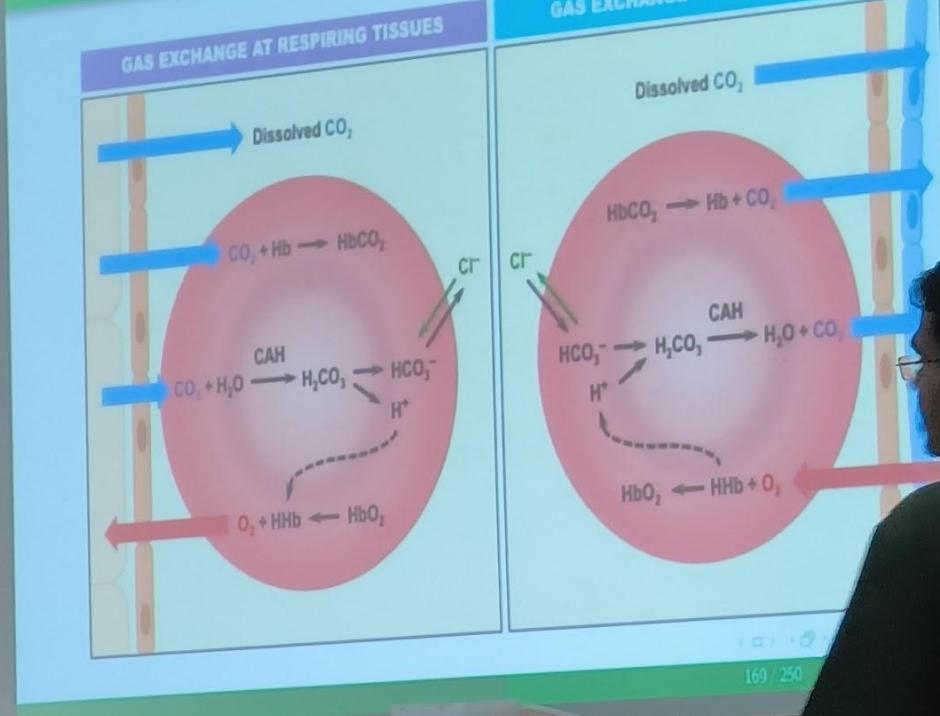
164 / 250



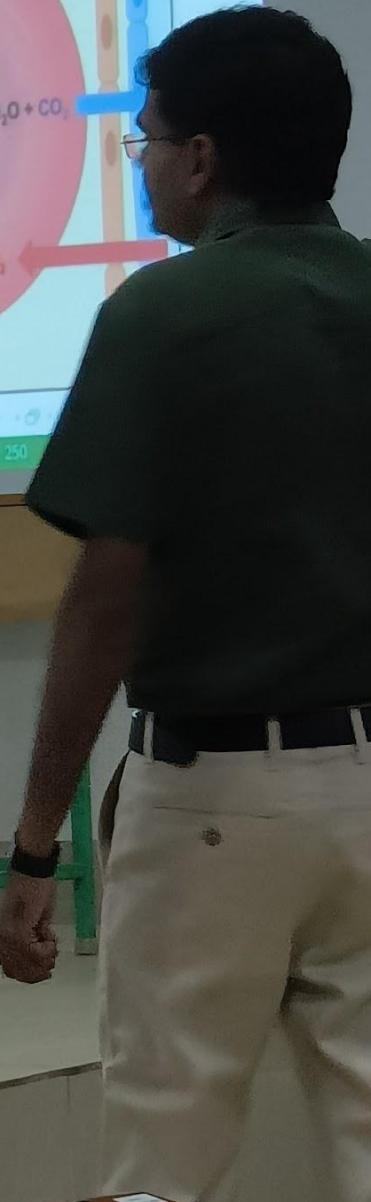
## Identifying cooperativity in enzyme catalysis



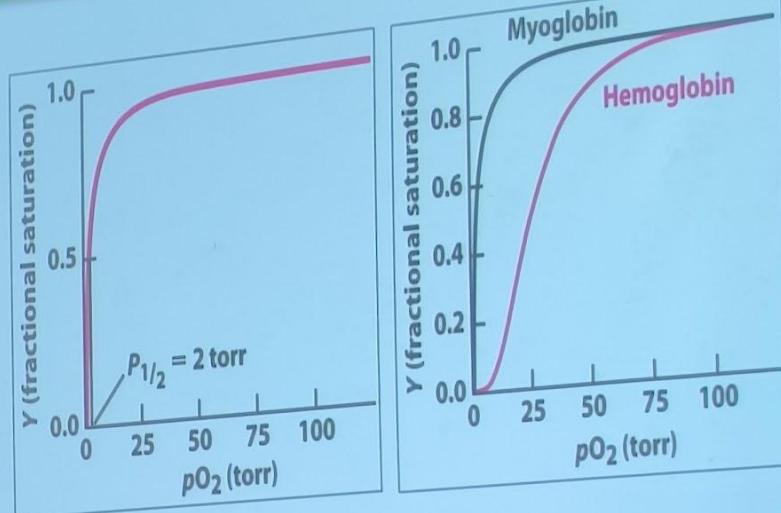
## Binding of oxygen to haemoglobin and myoglobin



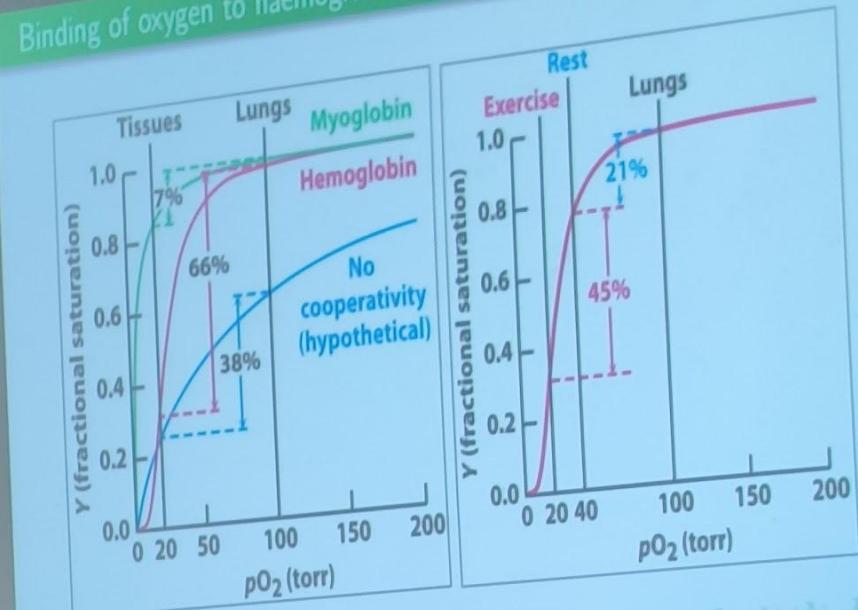
169 / 250



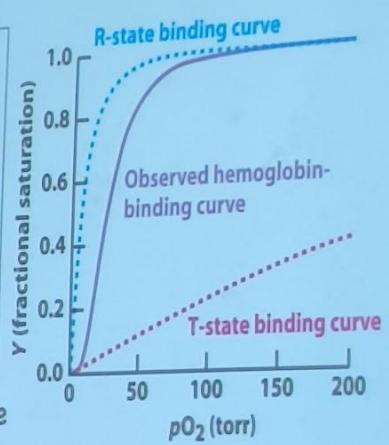
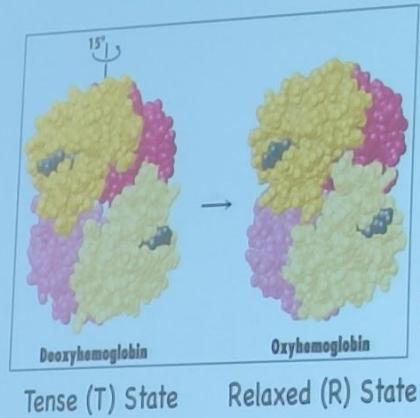
## Binding of oxygen to haemoglobin and myoglobin



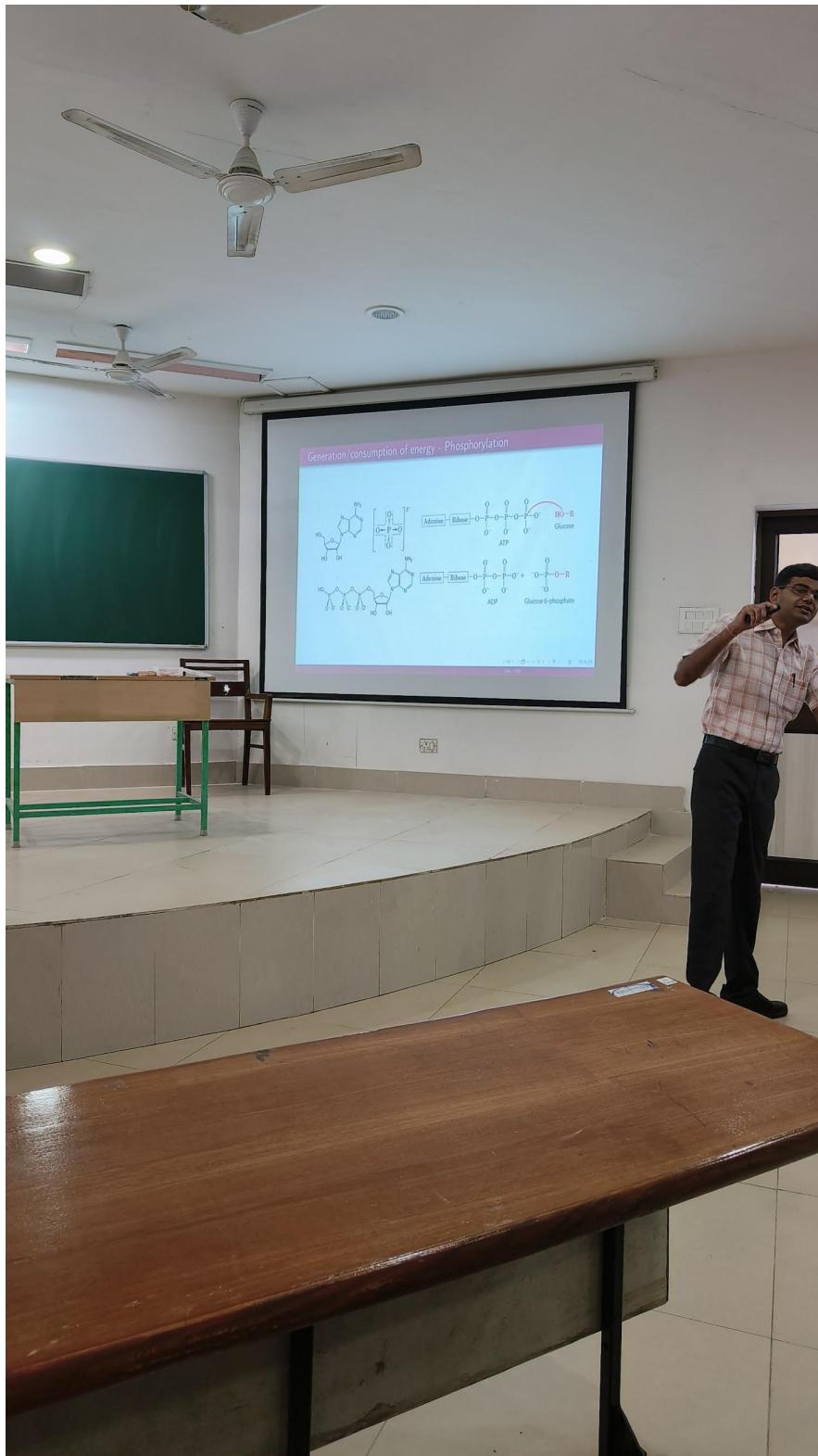
## Binding of oxygen to haemoglobin and myoglobin



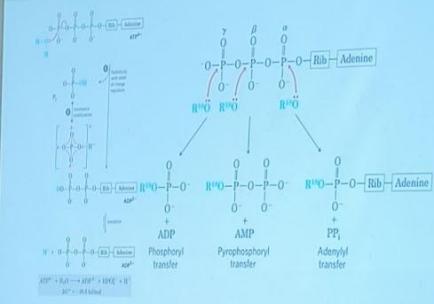
174 / 250



## REACTION MECHANISM (31 OCT 2022)

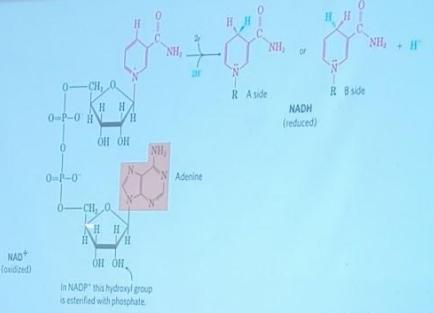


### Generation/consumption of energy - Phosphorylation

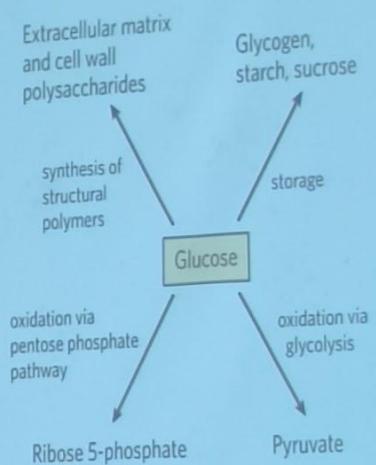
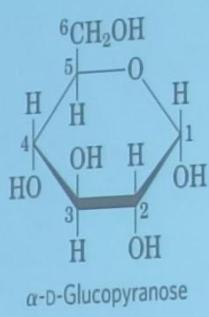
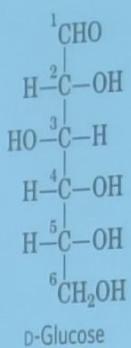


145 / 250

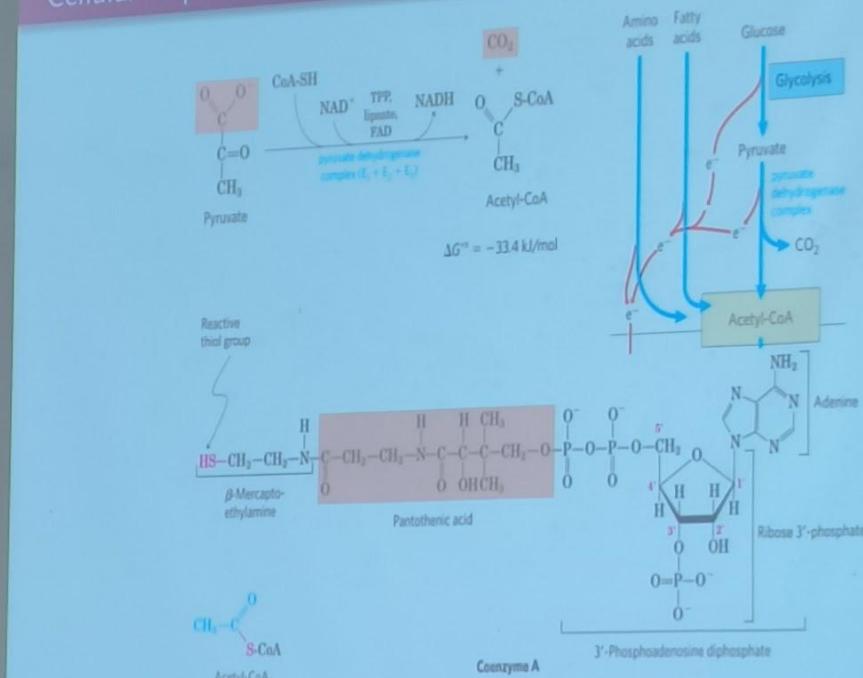
### Generation/consumption of energy - Oxidation/reduction



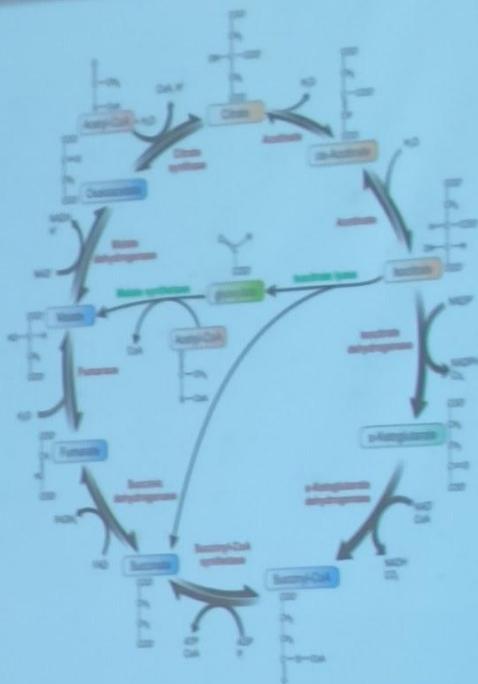
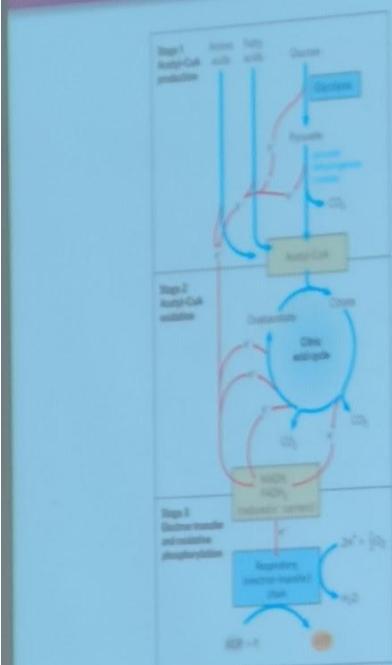
10 / 20



## Cellular respiration - Acetyl coenzyme formation

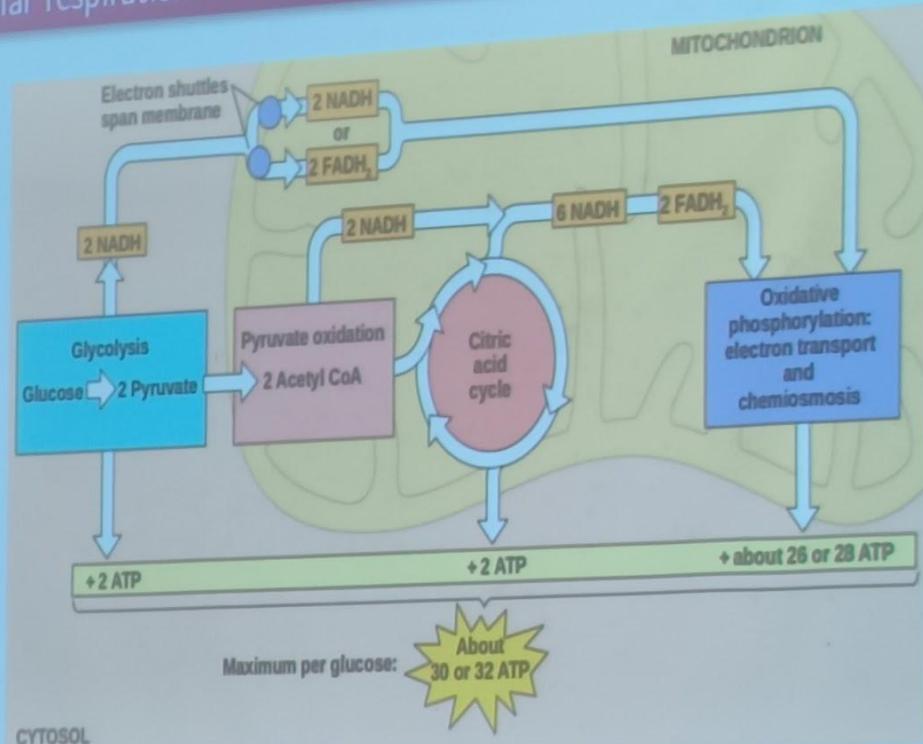


## Cellular respiration - Citric acid or Krebs or TCA cycle



291 / 292

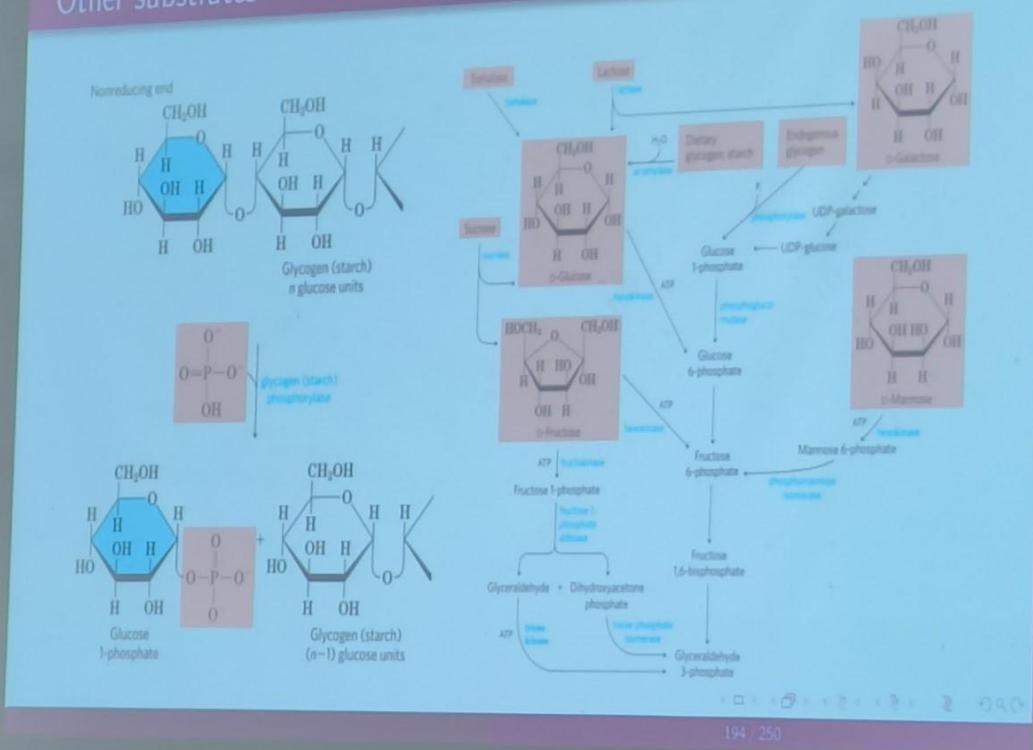
## Cellular respiration - Overall energy generation



## Cellular respiration - Overall energy generation

Reaction	Number of ATP or reduced coenzyme directly formed	Number of ATP ultimately formed
Glucose → glucose 6-phosphate	-1 ATP	-1
Fructose 6-phosphate → fructose 1,6-bisphosphate	-1 ATP	-1
2 Glyceraldehyde 3-phosphate → 2 1,3-bisphosphoglycerate	2 NADH	3 or 5
2 1,3-Bisphosphoglycerate → 2 3-phosphoglycerate	2 ATP	2
2 Phosphoenolpyruvate → 2 pyruvate	2 ATP	2
2 Pyruvate → 2 acetyl-CoA	2 NADH	5
2 Isocitrate → 2 α-ketoglutarate	2 NADH	5
2 α-Ketoglutarate → 2 succinyl-CoA	2 NADH	5
2 Succinyl-CoA → 2 succinate	2 ATP (or 2 GTP)	2
2 Succinate → 2 fumarate	2 FADH <sub>2</sub>	3
2 Malate → 2 oxaloacetate	2 NADH	5
Total		30-32

## Other substrates



Lack of glucose and inefficiency of glucose processing

## KETONE BODIES

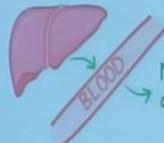
PLAN A  
GENERATE ENERGY

CARBOHYDRATES  
FATS  
PROTEINS

PLAN B  
ALTERNATIVE FUEL SOURCE

\* PRODUCED by LIVER MITOCHONDRIA  
USING ACETYL-CoA

PHYSIOLOGICAL STATES  
PATHOLOGICAL STATES

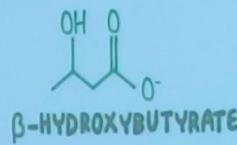


BLOOD  
MAJORITY  
of CELLS

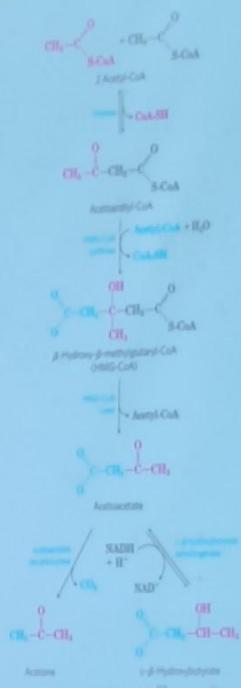
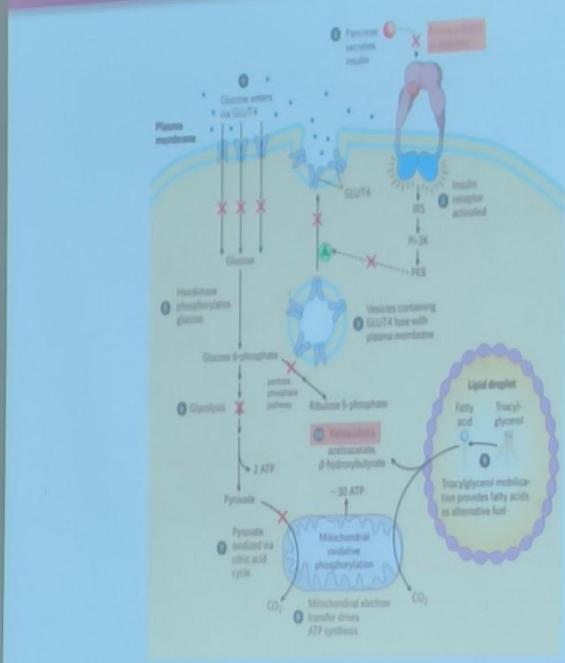
RECONVERTED into  
ACETYL-CoA



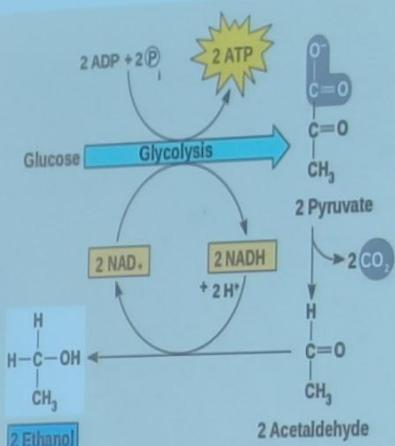
PRODUCE ATP



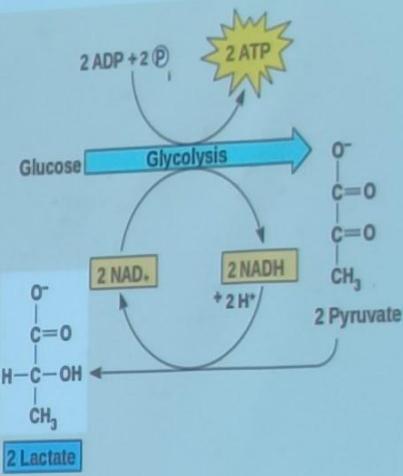
## Lack of glucose and inefficiency of glucose processing



## Pyruvate utilisation

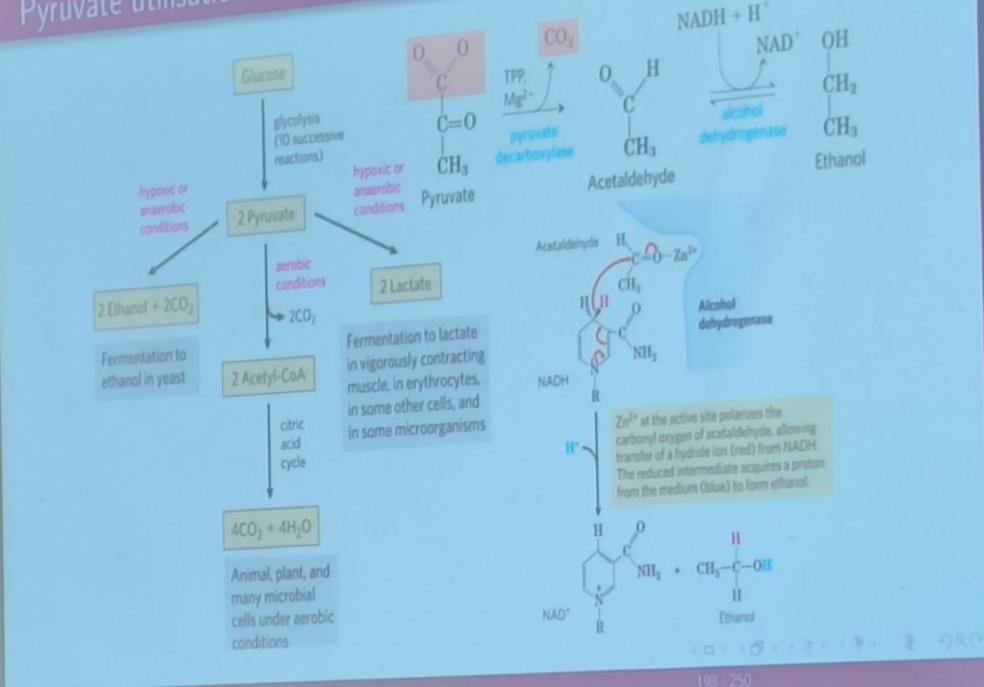


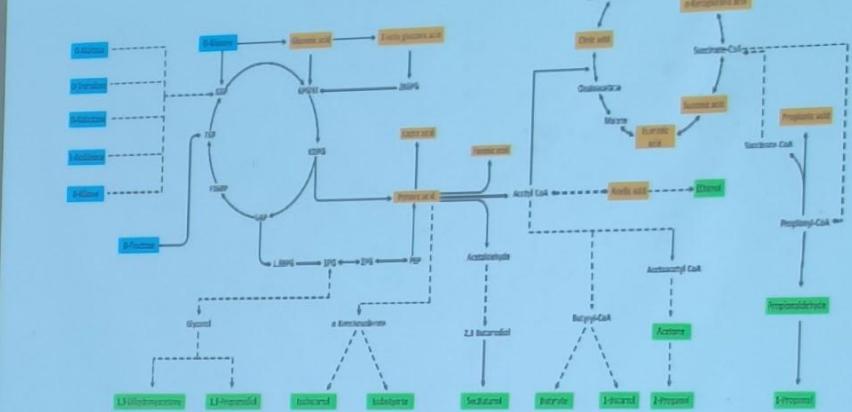
(a) Alcohol fermentation

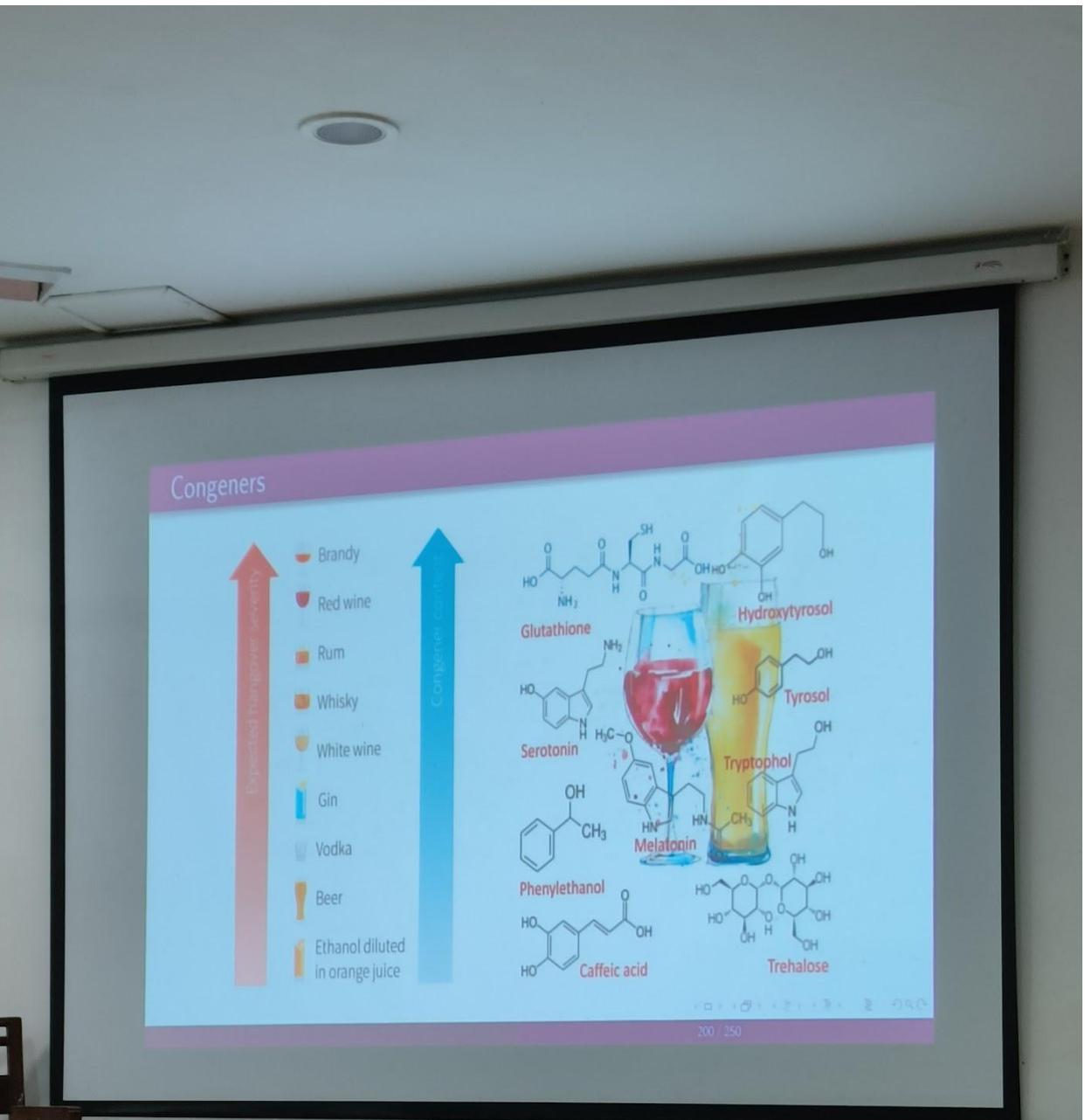


(b) Lactic acid fermentation

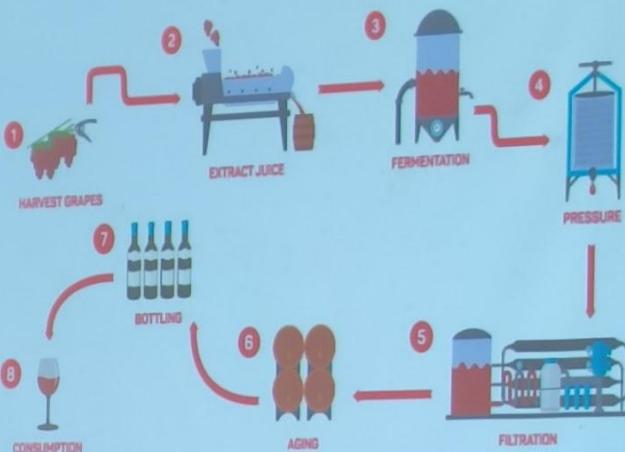
## Pyruvate utilisation





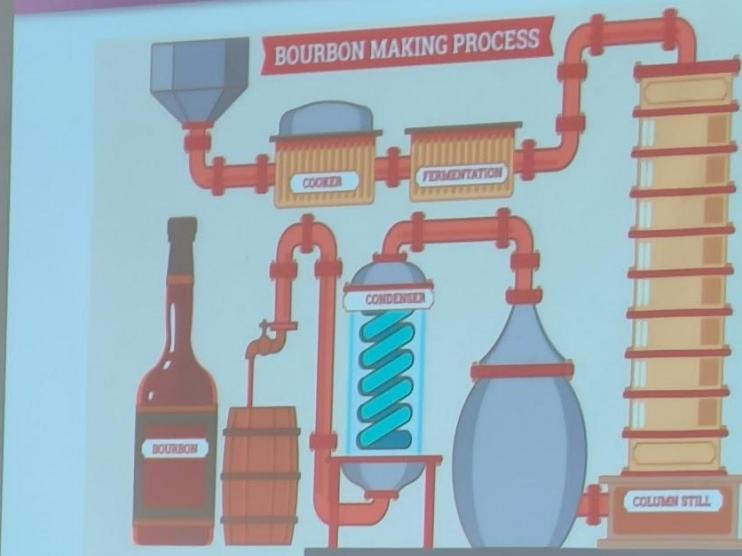


## Wine vs Whiskey vs Vodka



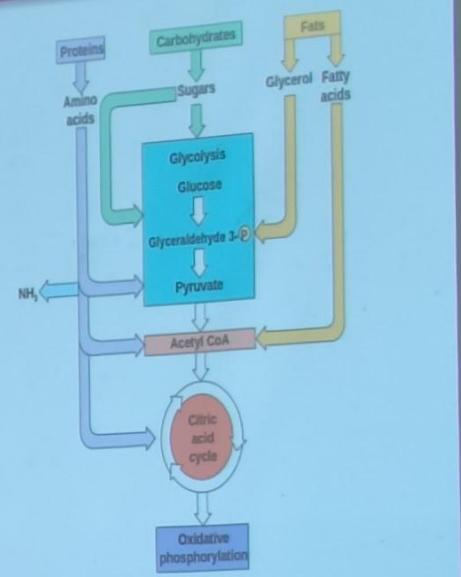
201 / 250

## Wine vs Whiskey vs Vodka

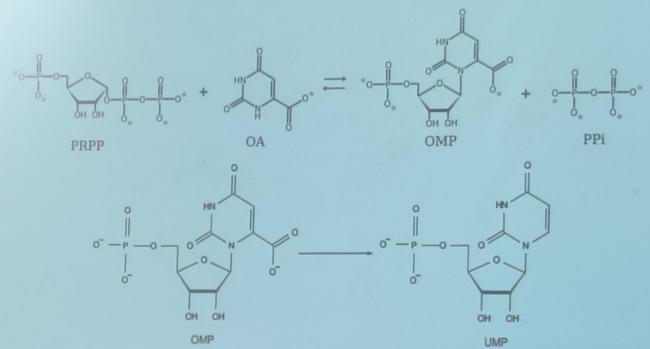


202 / 250

## Metabolism of different food sources



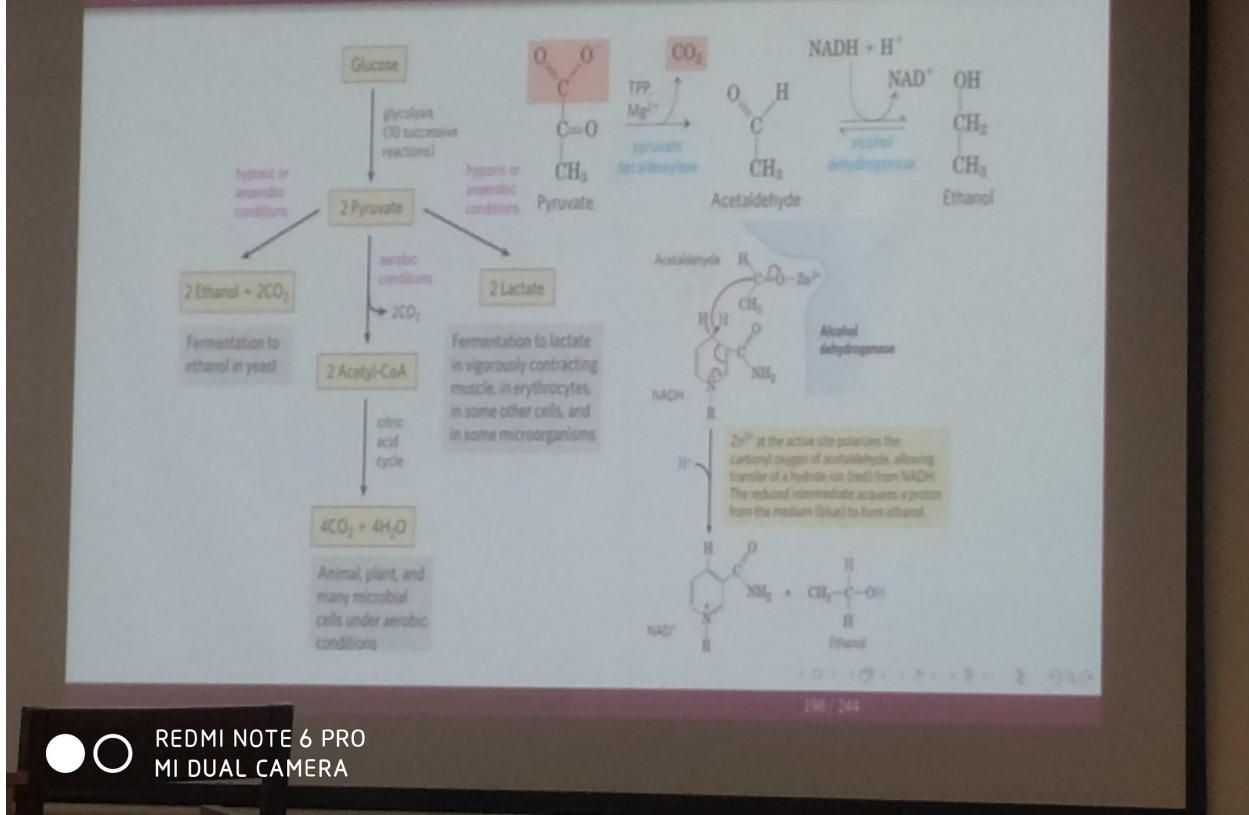
### Bimolecular reactions



132 / 250

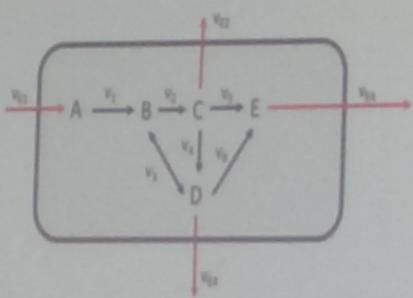
07-NOV-2022

## Pyruvate utilisation



REDMI NOTE 6 PRO  
MI DUAL CAMERA

## Metabolic flux analysis - A test model



Intracellular reaction rates:

$$v_1 : A \rightarrow B \quad (46)$$

$$v_2 : B \rightarrow C \quad (47)$$

$$v_3 : B \leftrightarrow D \quad (48)$$

$$v_4 : C \rightarrow D \quad (49)$$

$$v_5 : C \rightarrow E \quad (50)$$

$$v_6 : D \rightarrow E \quad (51)$$

$$v_{E1} : A \rightarrow \text{out} \quad (42)$$

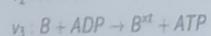
$$v_{E2} : B \rightarrow \text{out} \quad (43) \quad v_i: \text{intracellular fluxes}$$

$$v_{E3} : C \rightarrow \text{out} \quad (44) \quad v_E: \text{exchange fluxes}$$

$$v_{E4} : D \rightarrow \text{out} \quad (45) \quad *E. coli: 1136 metabolites; 2551 reactions$$



### Degree of freedom analysis



- ① Draw the network for the above-mentioned system
- ② Write the flux balance equations
- ③ Develop different matrices and write the relationships among them at steady-state
- ④ Carry out degree of freedom analysis
- ⑤ Generalise the degree of freedom analysis hence done to come up with the rules for determinacy of the system



REDMI NOTE 6 PRO  
MI DUAL CAMERA

## Handling under-determined systems - Linear programming

$$\begin{array}{ll}\text{max/min} & Z = \text{obj} \cdot \nu^T \\ \text{s.t.} & S \cdot \nu = 0 \\ & S^* \cdot \nu_i \leq b \\ & l b_i \leq \nu \leq u b_i\end{array}$$

$$\begin{array}{ll}\text{max} & Z = [1 \ 1] [\nu_1 \ \nu_2]^T \\ & \nu_1 + 2\nu_2 \leq 8 \\ & 0 \leq \nu_1 \leq 4 \\ & 0 \leq \nu_2 \leq 4\end{array}$$

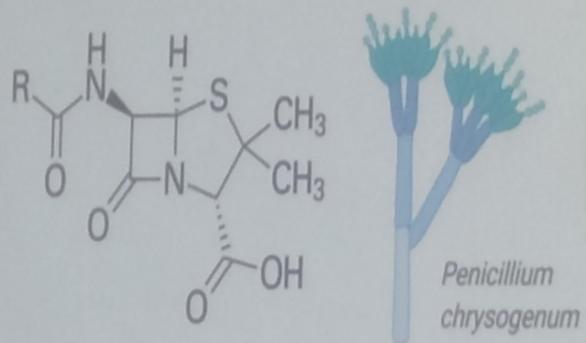
### Common objective functions

- Maximisation of biomass yield
- Maximisation of growth rate
- Maximisation of ATP yield per flux
- Minimisation of glucose consumption



REDMI NOTE 6 PRO  
MI DUAL CAMERA

Example - 1: Penicillin from *Penicillium chrysogenum*

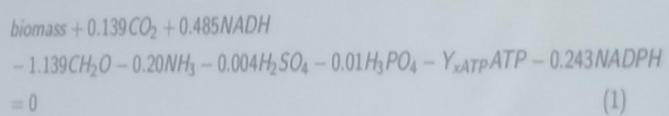


REDMI NOTE 6 PRO  
MI DUAL CAMERA

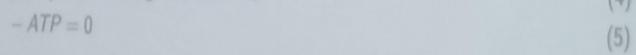
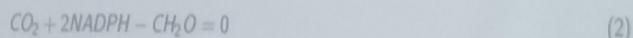
... 220 / 244

### Example - 1: Metabolic model for *Penicillium chrysogenum*

#### Overall stoichiometry



#### Individual reactions



Write the above set of equations as a matrix equation of the form:

$$\underline{\underline{A}} \underline{\underline{S}} + \underline{\underline{B}} \underline{\underline{P}} + \underline{\underline{C}} \underline{\underline{X}} + \underline{\underline{D}} \underline{\underline{I}} = 0$$



REDMI NOTE 6 PRO  
MI DUAL CAMERA

204 204

### Example - 2: Metabolic model for lactic acid bacteria

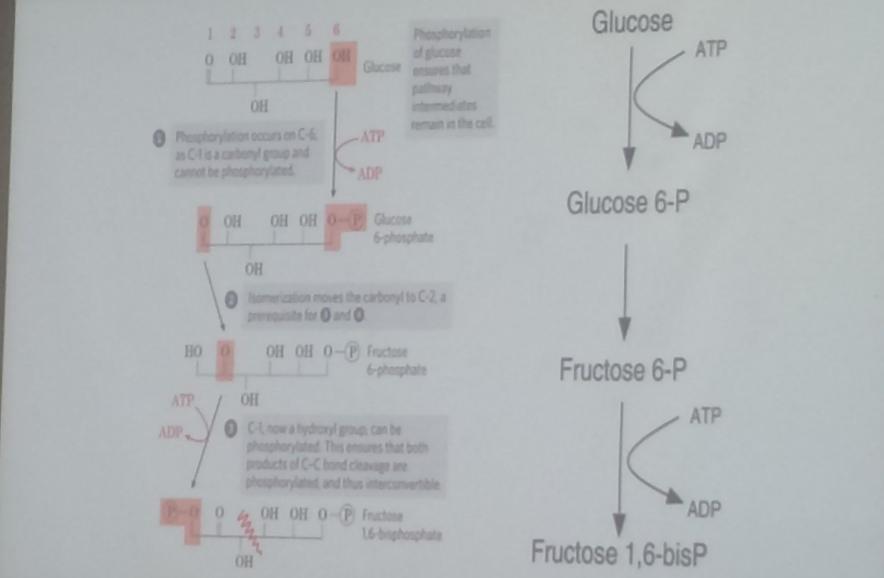
$$\begin{bmatrix} -0.5 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \end{bmatrix} [GLU] + \begin{bmatrix} 0 & 0 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} LAC \\ CO_2 \\ FOR \\ ACE \\ ETH \end{bmatrix} + \begin{bmatrix} 1 & 0 & 1 \\ -1 & 0 & -1 \\ -1 & 1 & 1 \\ -1 & 1 & 0 \\ 0 & -1 & 0 \\ 0 & -1 & -2 \end{bmatrix} \begin{bmatrix} PYR \\ ACOA \\ NADH \end{bmatrix} = \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \end{bmatrix}$$

- ① Using the above stoichiometry, write the pseudo-state-state balances for the intermediates in matrix equation form
- ② If pyruvate, lactate and acetate rates are non-measurable while  $CO_2$ , acetate and ethanol rates are measurable, express non-measurable rates in terms of measurable rates from the previous result



REDMI NOTE 6 PRO  
MI DUAL CAMERA

## Dynamical flux balance analysis: Glycolytic oscillations



229 / 244



REDMI NOTE 6 PRO  
MI DUAL CAMERA

## Dynamical equations for modeling glycolytic oscillations

$$\frac{d[S_1]}{dt} = v_1 - k_1[S_1][E - S_2^n] + k_{-1}[S_1 - E - S_2^n] \quad (10)$$

$$\frac{d[S_2]}{dt} = k_2[S_1 - E - S_2^n] - nk_3[S_2^n][E] + nk_{-3}[E - S_2^n] - v_2[S_2] \quad (11)$$

$$\frac{d[E - S_2^n]}{dt} = -k_1[S_1][E - S_2^n] + (k_{-1} + k_2)[S_1 - E - S_2^n] + k_3[S_2^n][E] - k_{-3}[E - S_2^n] \quad (12)$$

$$\frac{d[S_1 - E - S_2^n]}{dt} = k_1[S_1][E - S_2^n] - (k_{-1} + k_2)[S_1 - E - S_2^n] \quad (13)$$



REDMI NOTE 6 PRO  
MI DUAL CAMERA