BCHE2030 Tutorial 5

Group

Content

Enzyme types

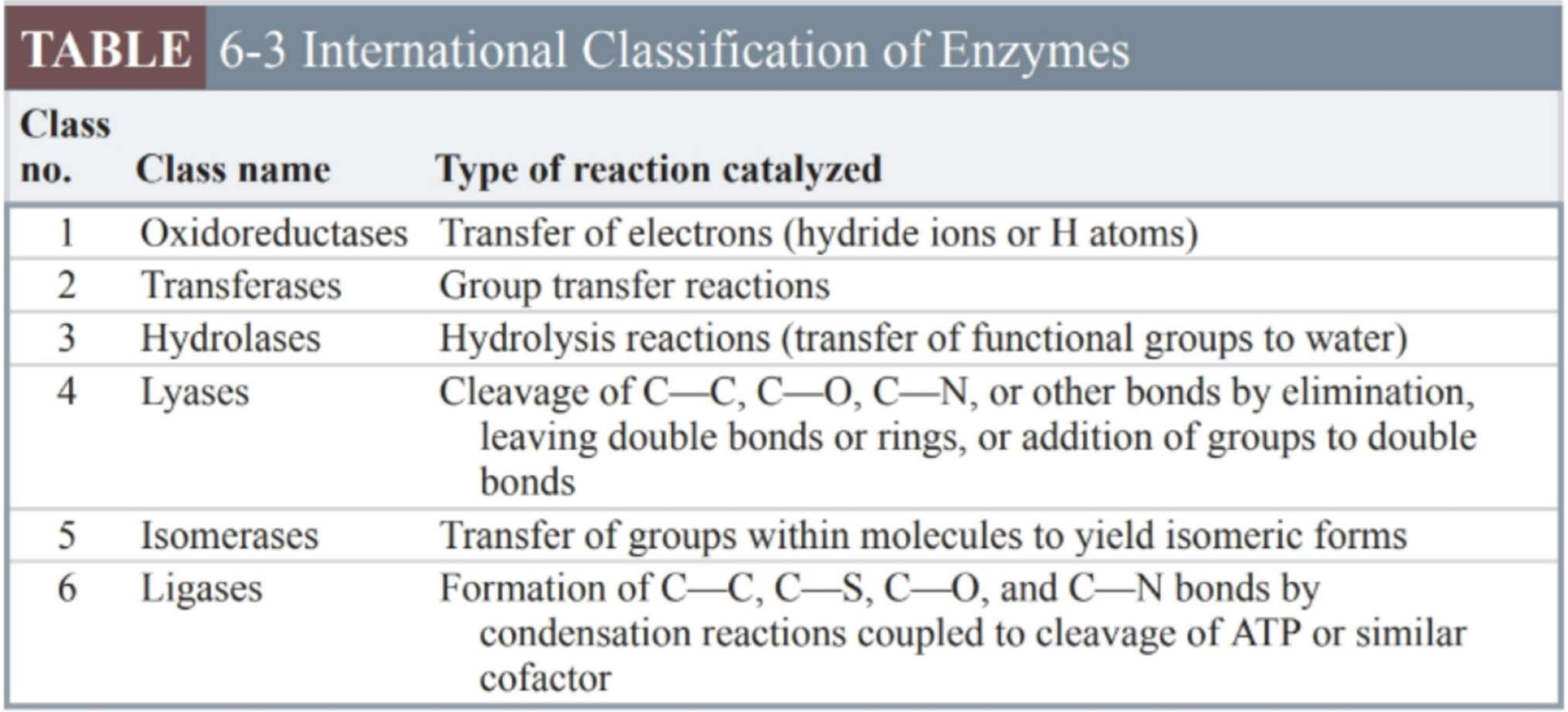


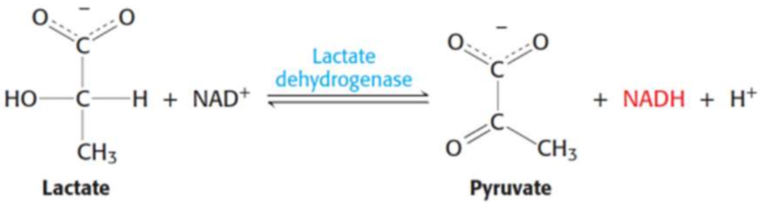
TABLE 6-3 International Classification of Enzymes

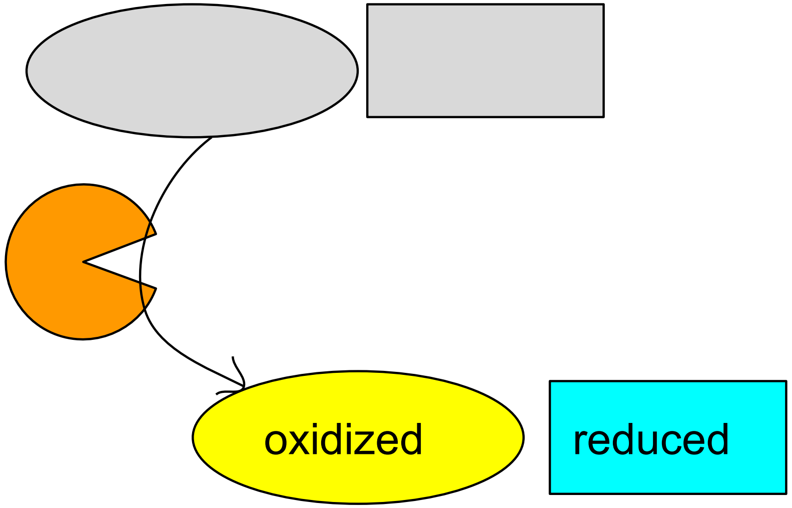
Class no.	Class name	Type of reaction catalyzed
1	Oxidoreductases	Transfer of electrons (hydride ions or H atoms)
2	Transferases	Group transfer reactions
3	Hydrolases	Hydrolysis reactions (transfer of functional groups to water)
4	Lyases	Cleavage of C—C, C—O, C—N, or other bonds by elimination, leaving double bonds or rings, or addition of groups to double bonds
5	Isomerases	Transfer of groups within molecules to yield isomeric forms
6	Ligases	Formation of C—C, C—S, C—O, and C—N bonds by condensation reactions coupled to cleavage of ATP or similar cofactor

Enzyme types

1. **oxidoreductases**: oxidation-reduction reaction

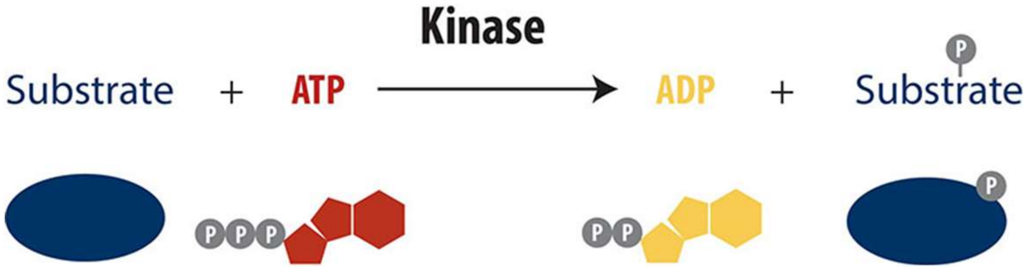
   e.g. lactate dehydrogenase (DH), alcohol DH

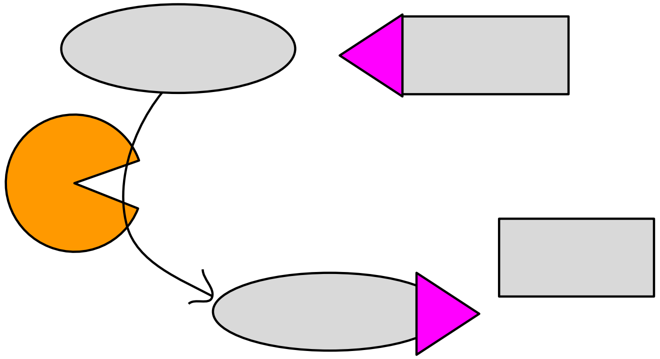




1. **transferases**: group transfer

   e.g. kinases, coenzyme A transferases

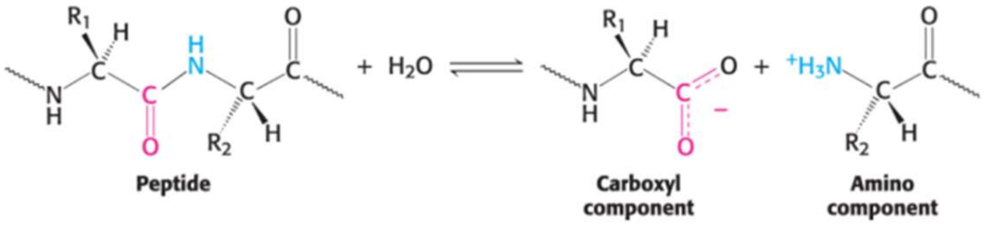




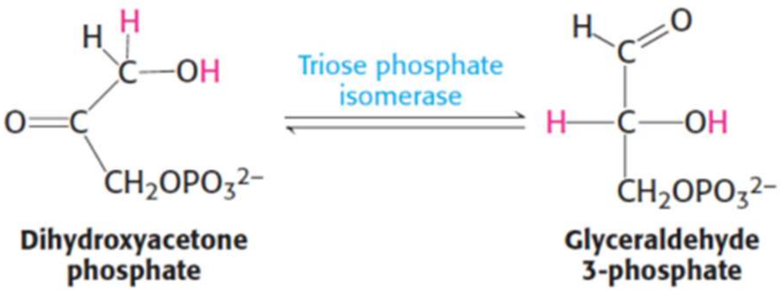
Enzyme types

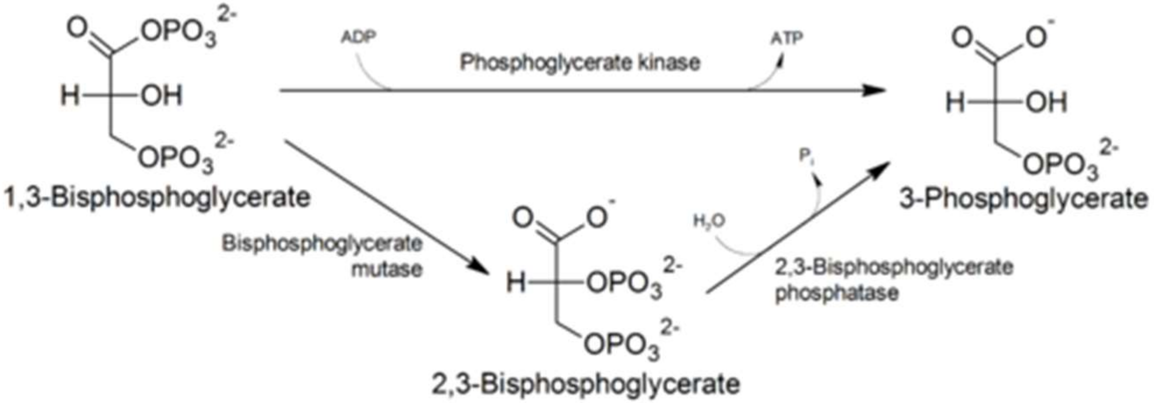
3. **hydrolases**: hydrolysis reaction

e.g. chymotrypsin, amylase, lipase



5. **isomerases**: isomerization (isomers such as geometric isomers or optical isomers)
e.g. triose phospahte isomerases, biphosphoglycerate mutase
(recall glycolysis and Hb)

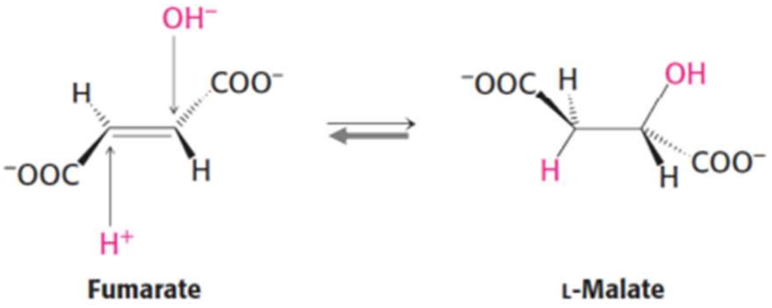


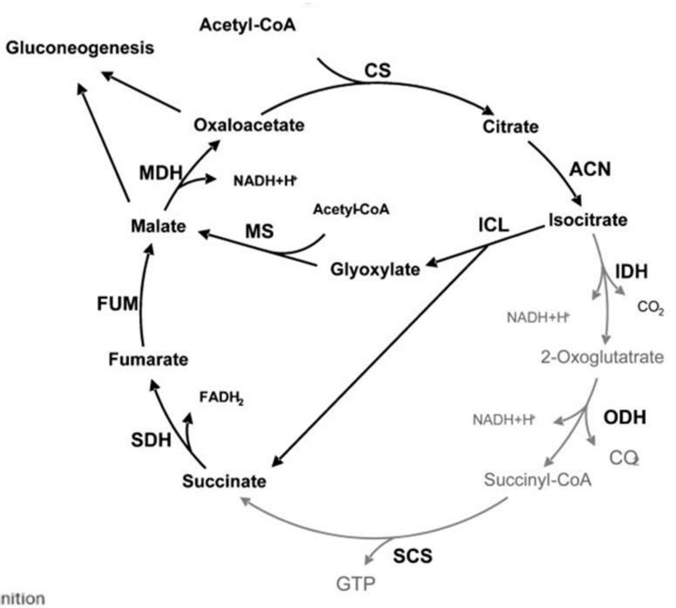


Enzyme types

4. **lyases**: cleaving bonds by means other than **hydrolysis** or **oxidation**

e.g. fumarase in TCA cycle, isocitrate lyase in glycosylate cycle

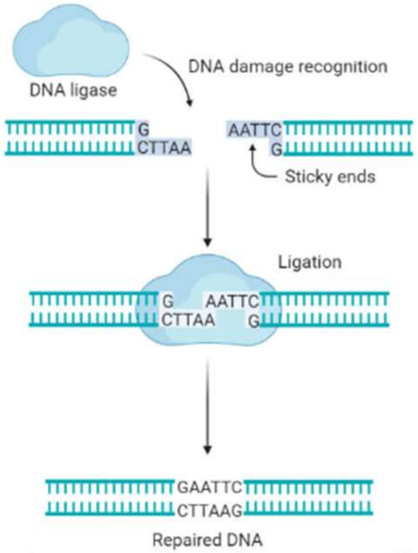


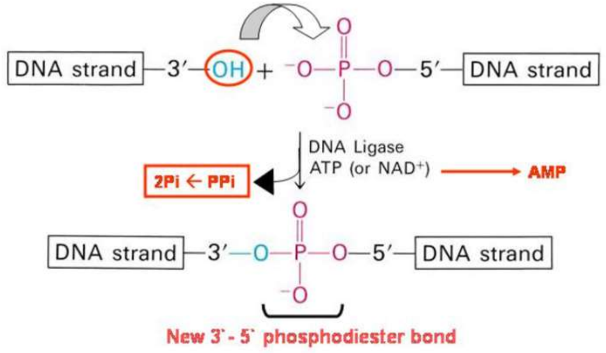


6. **ligase**: forming bonds with hydrolysis of ATP

e.g. Aminoacyl-tRNA synthetase, DNA ligase

$$\text{Amino acid} + \text{ATP} + \text{tRNA} \rightleftharpoons \text{aminoacyl-tRNA} + \text{AMP} + \text{PP}_i$$





(7. translocase, with the hydrolysis of ATP. e.g. ABC transporters, active transport)

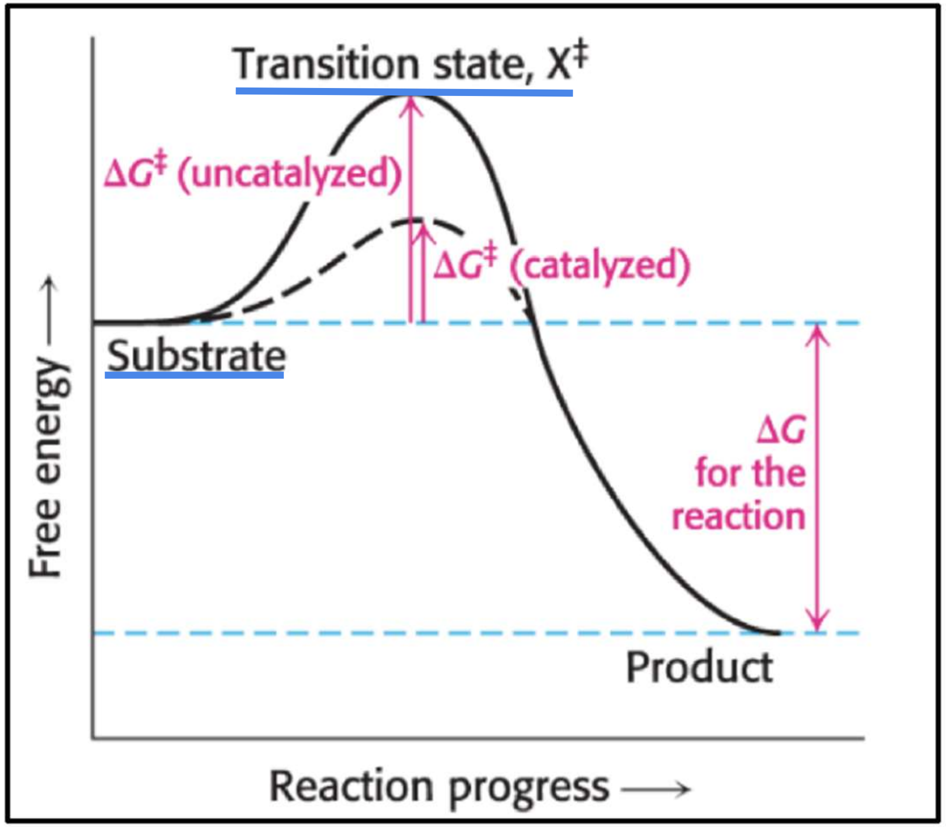
Enzyme speeds up reaction rate but doesn’t change equilibrium

- Enzyme lowers the **activation energy** (ΔG between substrate and transition state) so it speeds up the reaction.

change!

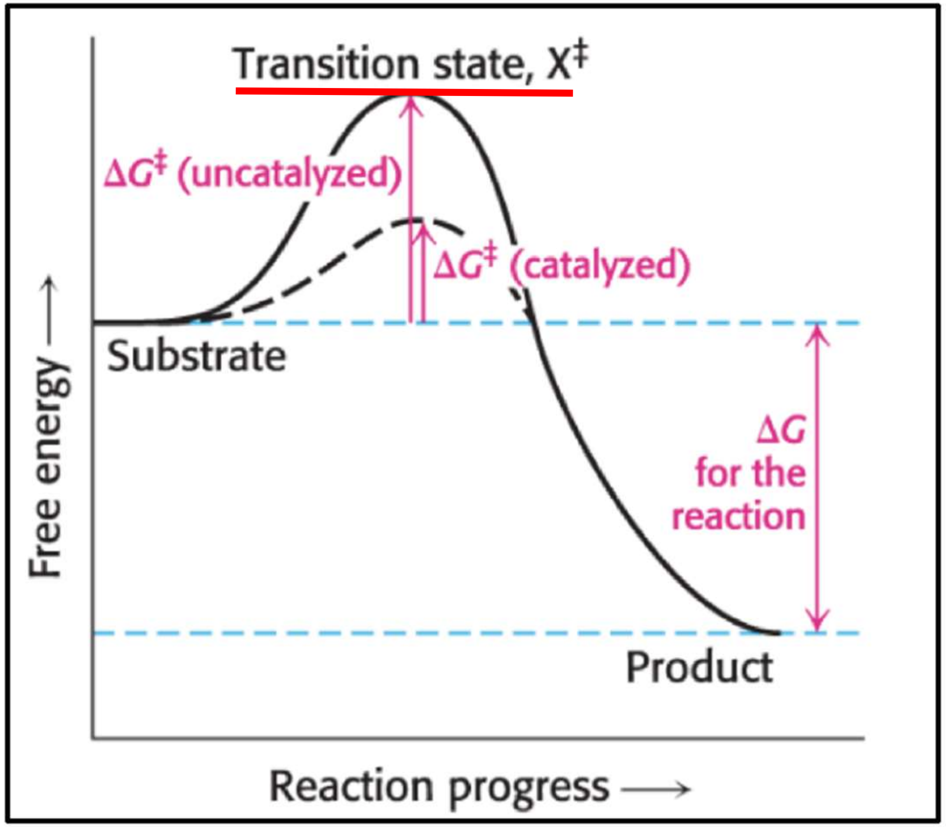
unchange!

- But enzyme doesn’t change the ΔG of the whole reaction, so it doesn’t change the equilibrium position.



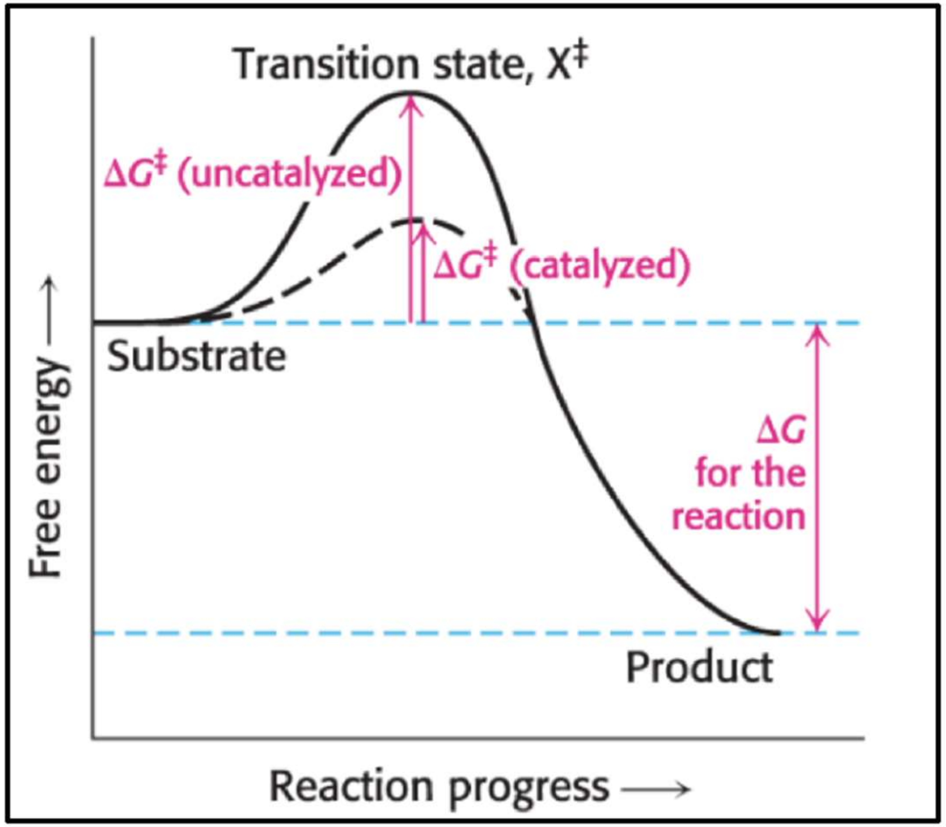
Enzyme speeds up reaction rate but doesn’t change equilibrium

- Transition state:
  - no longer the substrate, but is not yet the product
  - an instantaneous status
  - **highest free energy** (G)



Enzyme speeds up reaction rate but doesn’t change equilibrium

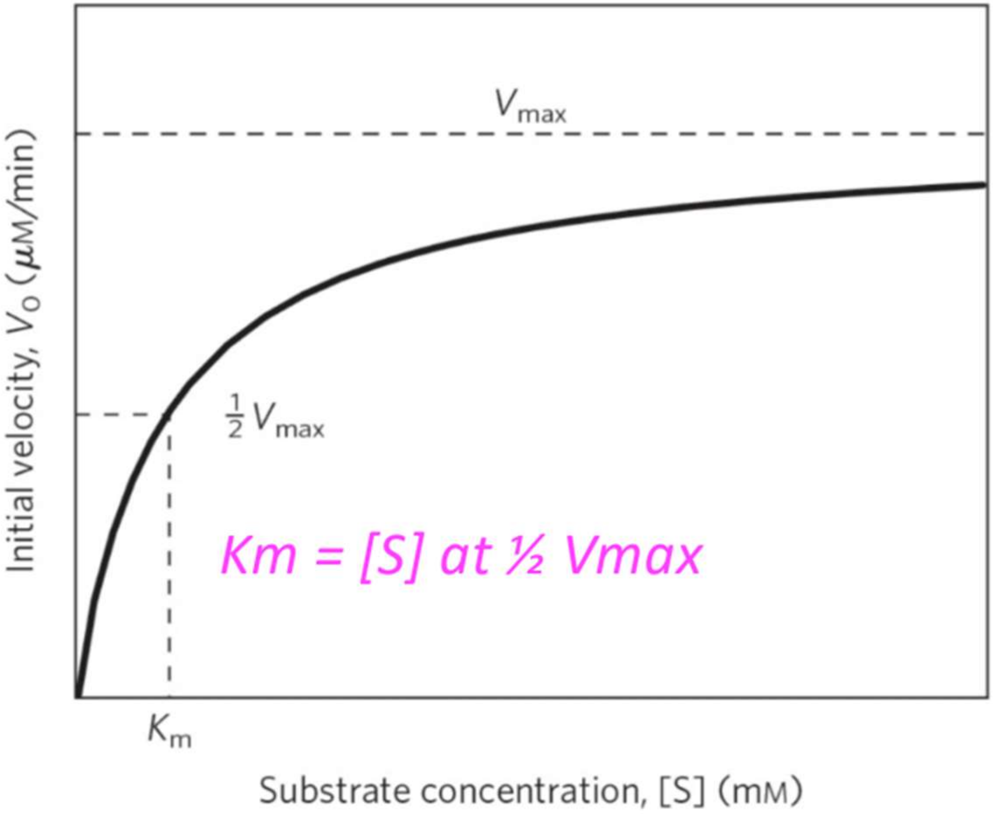
- **Binding energy**:
  - The binding energy is the **free energy** that is released by the formation of **non-covalent** interactions **between substrate (but not product) and enzyme**.
  - = ΔG (uncatalyzed) – ΔG (catalyzed)
  - enzyme being complementary to the transition state -> the **maximum** binding energy is released at the formation of **transition state**, stabilize the transition state, catalyze the reaction to happen



Mechaelis-Menton Kinetics

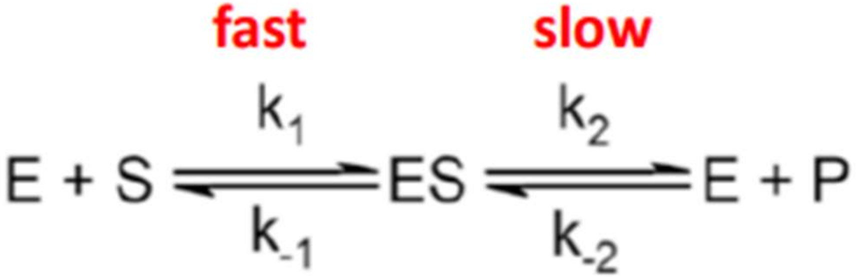
$$V_0 = \frac{V_{\max}[S]}{K_m + [S]}$$

- a measure in steady-state kinetics (single substrate reaction)
- Vo: initial velocity of reaction at certain [S]
- Vmax: velocity when **all enzyme binds with substrate**
- Km: reflects **affinity of enzyme** towards a **specific substrate**
- Km depends on type of substrate and enzyme



Break Down Different Constants

- **Km=(k2+k-1)/k1**, not exactly equals to binding affinity (k-1/k1)
- **Vmax = k2[ES]**
- When [S] is high, all enzyme binds with substrate, [ES]=[E]
- **Vmax = k2[ES]=k2[E]**
- k-2 negligible in Mechaelis-Menton kintics

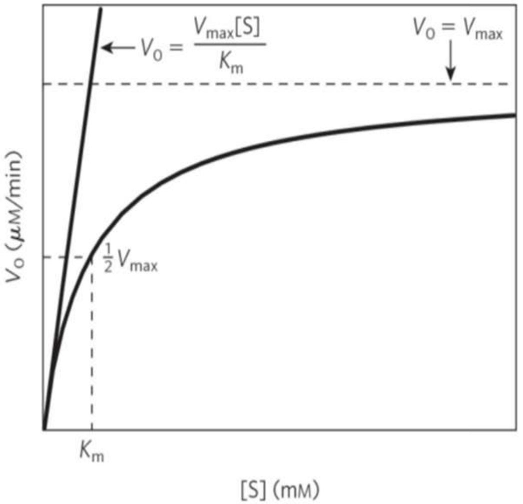


$$V_0 = \frac{k_{cat}[E_t][S]}{K_m + [S]}$$

Reading Mechaelis-Menton Equation

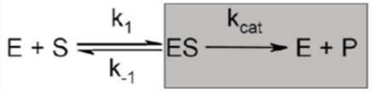
- Km: smaller Km=higher affinity
- kcat (turnover rate): no. of reaction process at catalytic site per unit time
- kcat/Km: catalytic efficiency of an enzyme
- kcat/Km limited by k_1(diffusion of substrate)
- When [S] >> Km , Vo = kcat [Et](rate not depends on substrate)
- When [S] << Km , Vo = kcat [Et] [S] / Km (first order)

Km=(k2+k-1)/k1



- When all enzymes are saturated with subst

[ES] = [Et] = total concentration of enzyme



$$V_{max} = k_{cat}[E_t]$$

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

$$\Rightarrow V_0 = \frac{k_{cat}[E_t][S]}{K_m + [S]}$$

Quiz 1

Which of the following statement(s) is/are INCORRECT?

A. Phosphotransferase is a kind of kinases
B. Lactate dehydrogenase requires coenzyme to catalyze the reaction
C. Hydrolase is present in saliva
D. Ligase is the only type of enzyme which involves hydrolysis
E. Fumarate is oxidized to malate by fumarase
F. ATP is used in the formation of phosphodiester bond by DNA ligase

Quiz 1

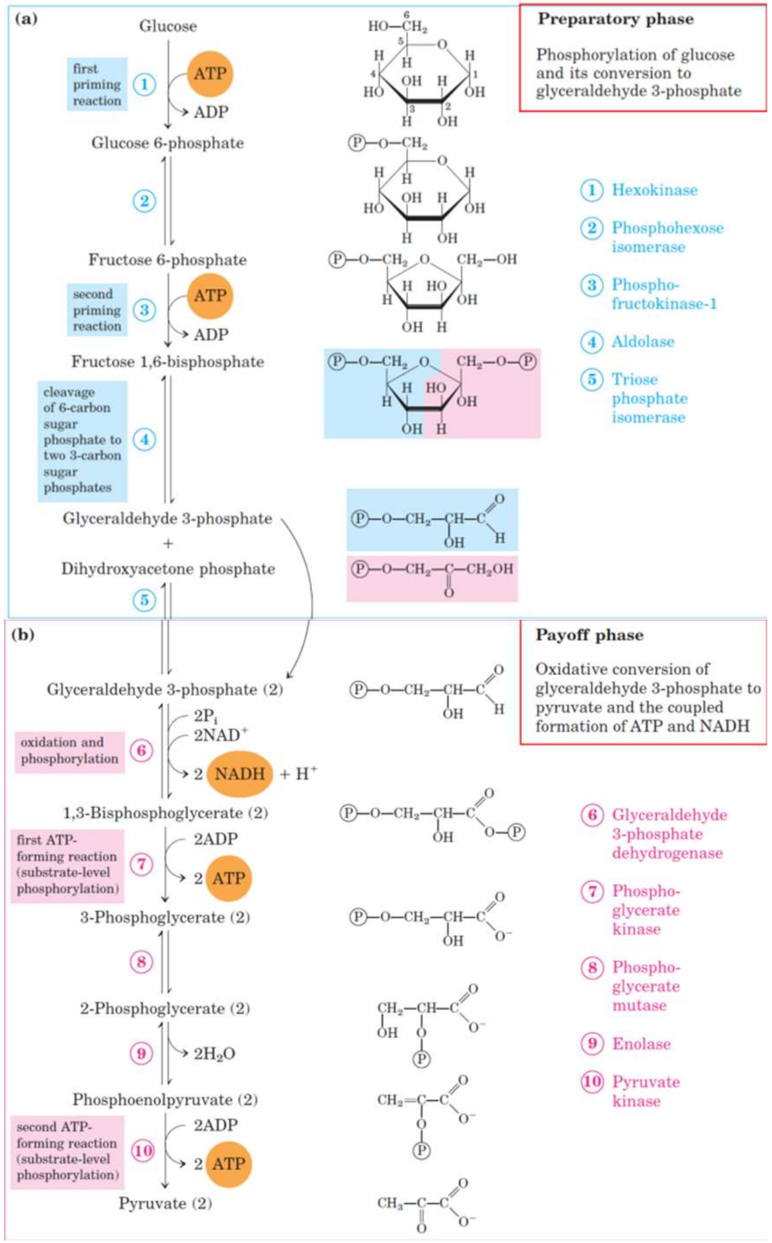
Which of the following statement(s) is/are INCORRECT?

- **A. Phosphotransferase is a kind of kinases (kinase: transfer Pi from ATP)**
- B. Lactate dehydrogenase requires coenzyme to catalyze the reaction
- C. Hydrolase is present in saliva
- **D. Ligase is the only type of enzyme which involves hydrolysis**
- **E. Fumarate is oxidized to malate by fumarase**
- F. ATP is used in the formation of phosphodiester bond by DNA ligase

Glysolysis

understand

1. rate limiting steps
2. first committed step
3. Input/product of glycolysis



Glysolysis (rate limiting steps)

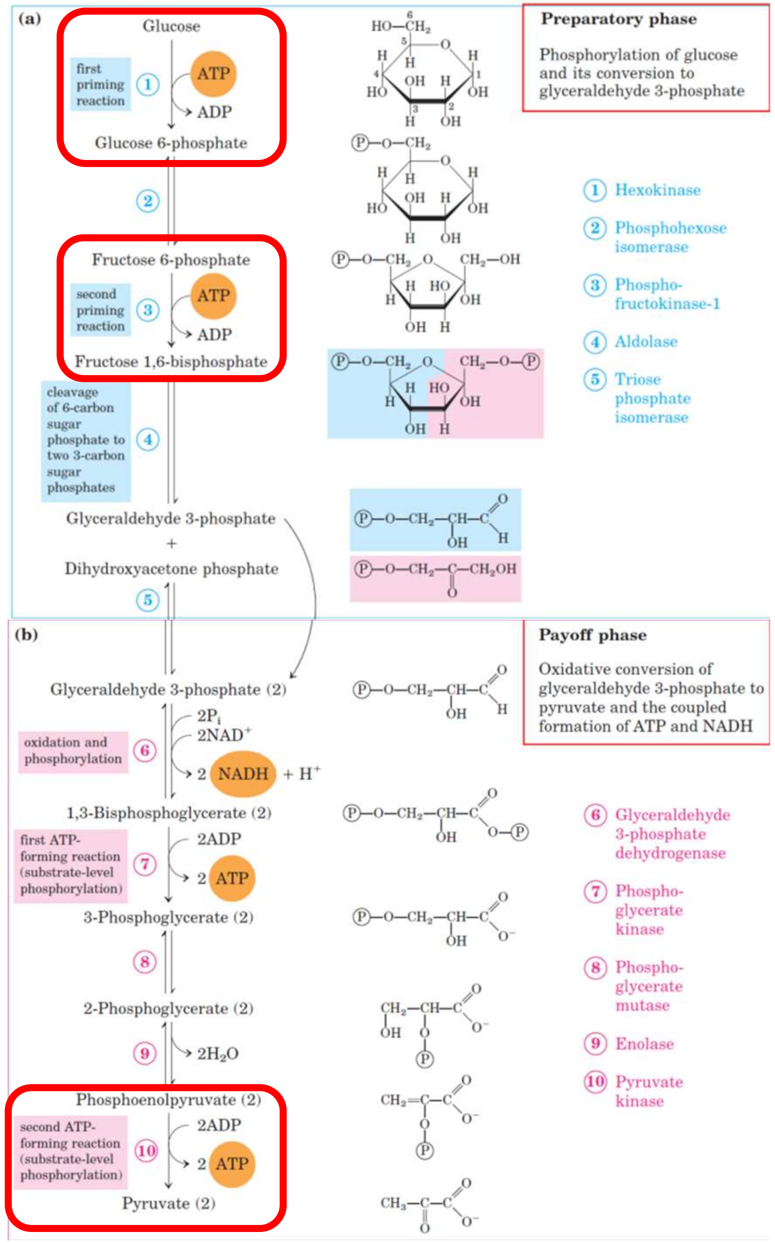
Step1, Step3, Step10

Allosteric regulation of enzyme

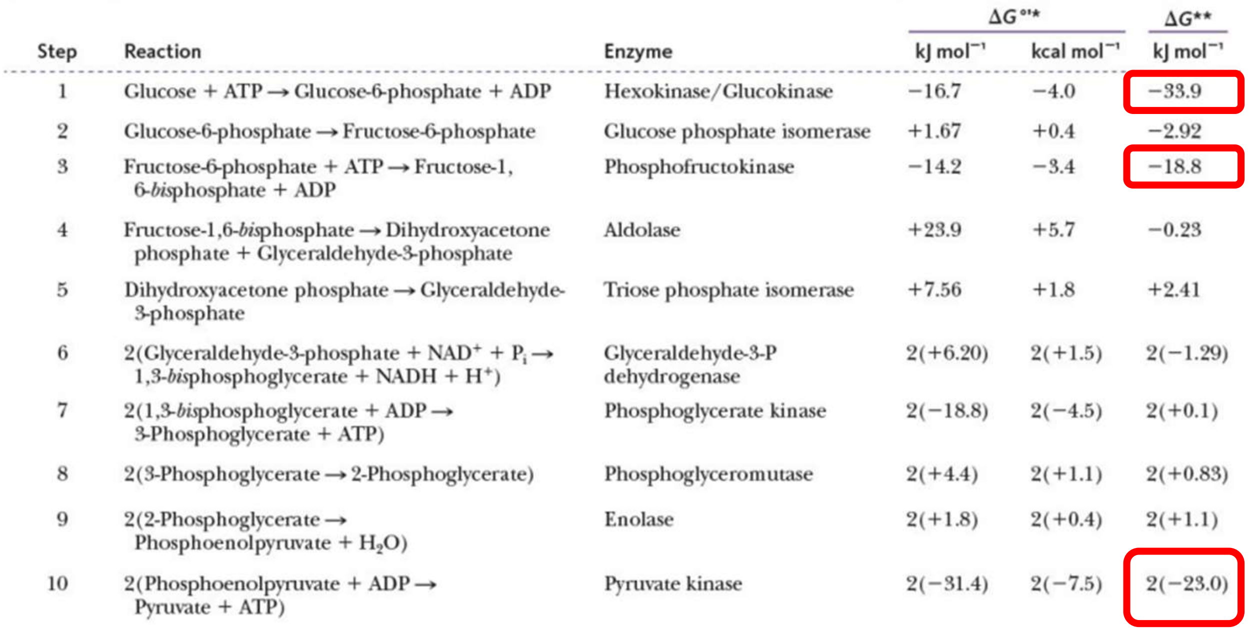
step1 **(hexokinase)** inhibit by G-6-P

step3 **(PFK-1)** inhibit by ATP

step10 **(pyruvate kinase)** inhibit by ATP

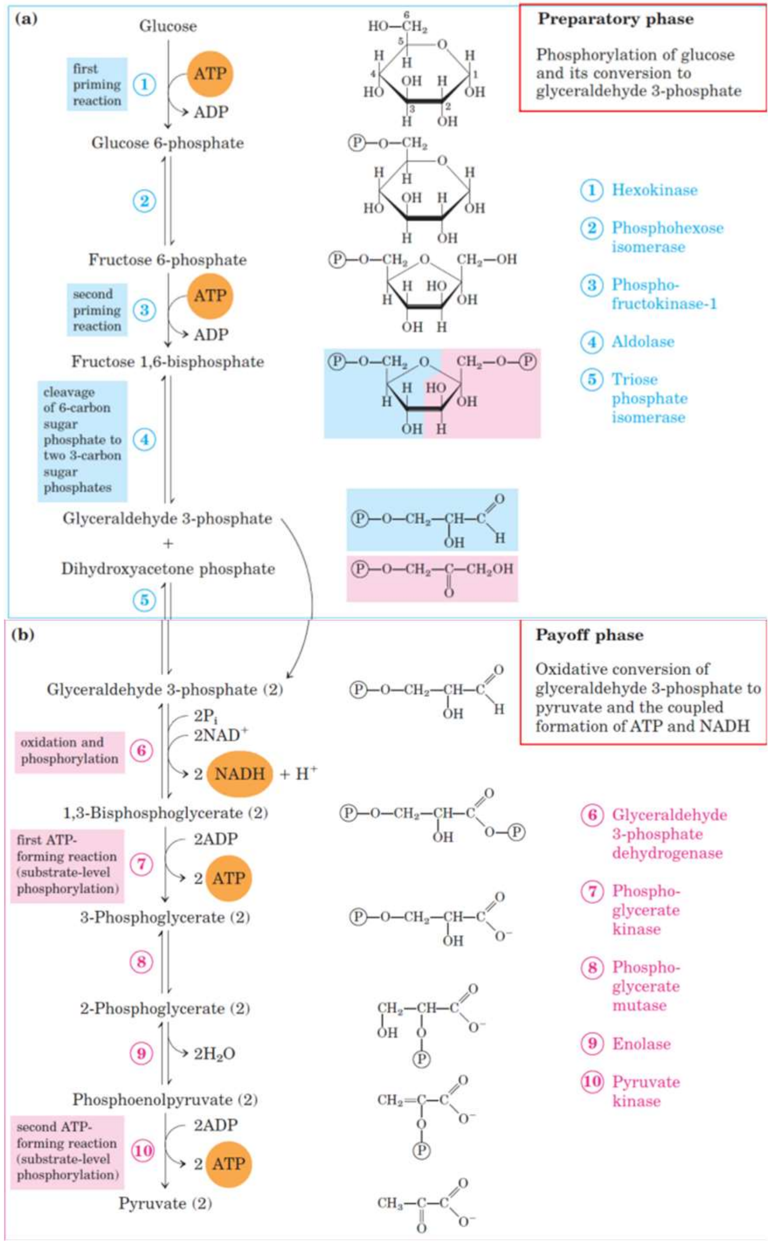


Glysolysis (rate limiting steps)



Step	Reaction	Enzyme	$\Delta G^{\circ\prime *}$ kJ mol^{-1}	$\Delta G^{\circ\prime *}$ kcal mol^{-1}	ΔG^{**} kJ mol^{-1}
1	Glucose + ATP → Glucose-6-phosphate + ADP	Hexokinase/Glucokinase	−16.7	−4.0	−33.9
2	Glucose-6-phosphate → Fructose-6-phosphate	Glucose phosphate isomerase	+1.67	+0.4	−2.92
3	Fructose-6-phosphate + ATP → Fructose-1,6-*bis*phosphate + ADP	Phosphofructokinase	−14.2	−3.4	−18.8
4	Fructose-1,6-*bis*phosphate → Dihydroxyacetone phosphate + Glyceraldehyde-3-phosphate	Aldolase	+23.9	+5.7	−0.23
5	Dihydroxyacetone phosphate → Glyceraldehyde-3-phosphate	Triose phosphate isomerase	+7.56	+1.8	+2.41
6	2(Glyceraldehyde-3-phosphate + NAD$^+$ + P_i → 1,3-*bis*phosphoglycerate + NADH + H$^+$)	Glyceraldehyde-3-P dehydrogenase	2(+6.20)	2(+1.5)	2(−1.29)
7	2(1,3-*bis*phosphoglycerate + ADP → 3-Phosphoglycerate + ATP)	Phosphoglycerate kinase	2(−18.8)	2(−4.5)	2(+0.1)
8	2(3-Phosphoglycerate → 2-Phosphoglycerate)	Phosphoglyceromutase	2(+4.4)	2(+1.1)	2(+0.83)
9	2(2-Phosphoglycerate → Phosphoenolpyruvate + H_2O)	Enolase	2(+1.8)	2(+0.4)	2(+1.1)
10	2(Phosphoenolpyruvate + ADP → Pyruvate + ATP)	Pyruvate kinase	2(−31.4)	2(−7.5)	2(−23.0)

Large free energy release - irreversible step



Glysolysis committed step

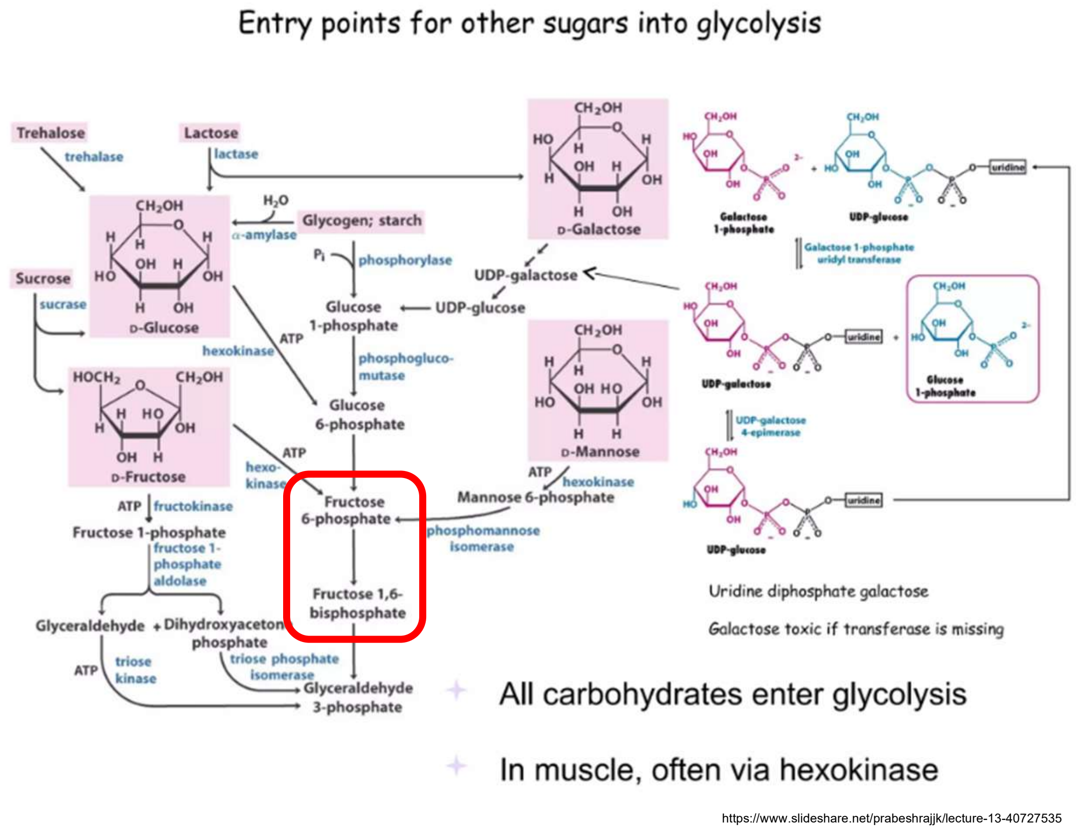
Step 3 (**PFK-1**)

It is committed to proceeding all the way to pyruvate

i.e. to completing glycolysis

(otherwise: G-6-P -> pantose phosphate pathway)

Sugar are “funnel” to F-6-P to process step 3



Entry points for other sugars into glycolysis

Uridine diphosphate galactose

Galactose toxic if transferase is missing

- All carbohydrates enter glycolysis
- In muscle, often via hexokinase

https://www.slideshare.net/prabeshrajjk/lecture-13-40727535

Glysolysis overview

The overall balance sheet show a net gain of ATP

$$\text{Glucose} + 2\text{ATP} + 2\text{NAD}^+ + 4\ \text{ADP} + 2\text{P}_i \rightarrow$$

$$2\ \text{pyruvate} + 2\text{ADP} + 2\text{NADH} + 2\text{H}^+ + 4\text{ATP} + 2\text{H}_2\text{O}$$

$$\text{Glucose} + 2\text{NAD}^+ + 2\text{ADP} + 2\text{P}_i \rightarrow$$

$$2\ \text{pyruvate} + 2\text{NADH} + 2\text{H}^+ + 2\text{ATP} + 2\text{H}_2\text{O}$$

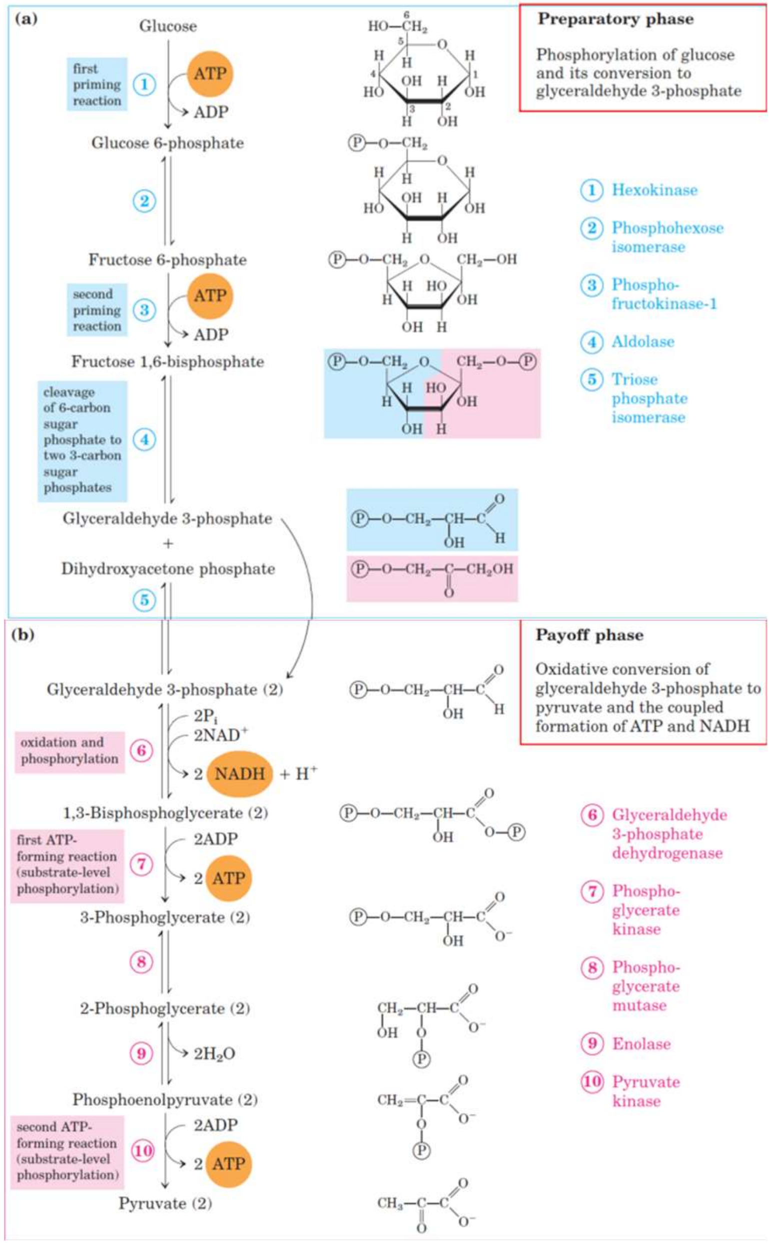
ATP consumption:

Step 1, step 3 = -2ATP

ATP pay off:

step 6 coupled with step 7, +2ATP +2 NADH

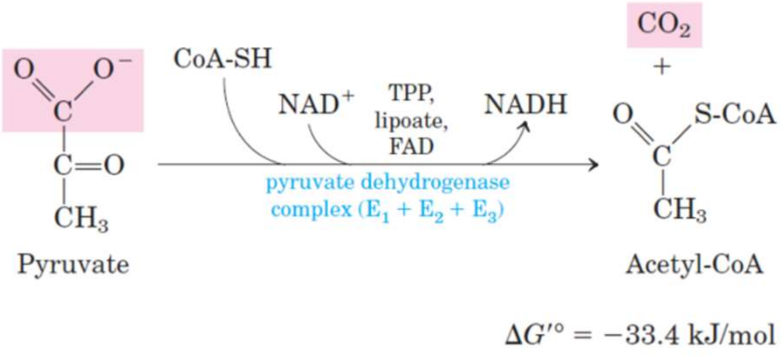
Step 10 +2ATP



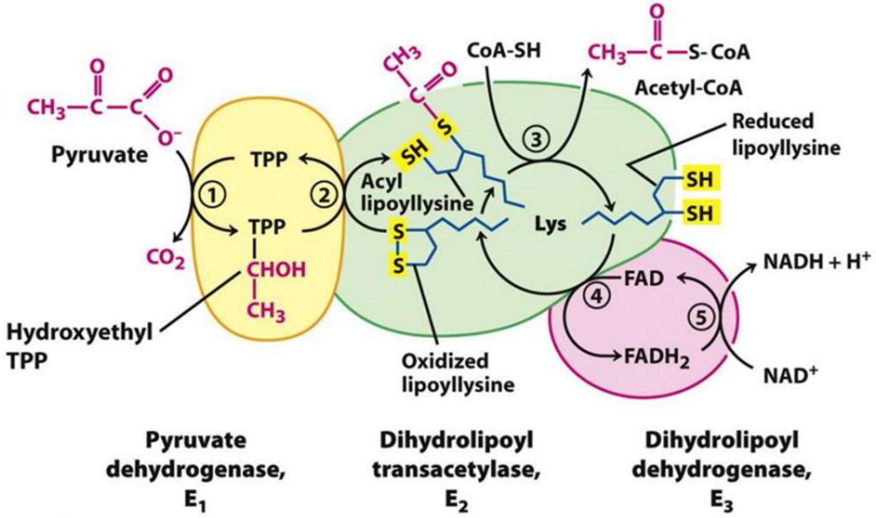
Glycolysis to TCA cycle

Pyruvate to Acetyl-CoA

- 5 coenzymes (Coenzyme A, NAD+, TPP, lipoate, FAD) are required
- Pyruvate dehyrogenase complex



TPP: thiamine pyrophospl
CoA-SH: Coenzyme A



Quiz 2

Which of the following glycolysis steps does not involve ATP-ADP coupled?

A. Step 1
B. Step 3
C. Step 6
D. Step 7
E. Step 10

Quiz 2

Which of the following glycolysis steps does not involve ATP/ADP coupled?

A. Step 1
B. Step 3
C. Step 6
D. Step 7
E. Step 10

Step 6 involve NAD+/NADH coupled

Step 6 has deltaG>0 and produces high energy compounds, but it couples with Step 7 (deltaG<0, highly favourable)!