



ALMA MATER STUDIORUM  
UNIVERSITÀ DI BOLOGNA

# CARBOHYDRATE METABOLISM – GLYCOLYSIS

**Prof. Michele Di Foggia**

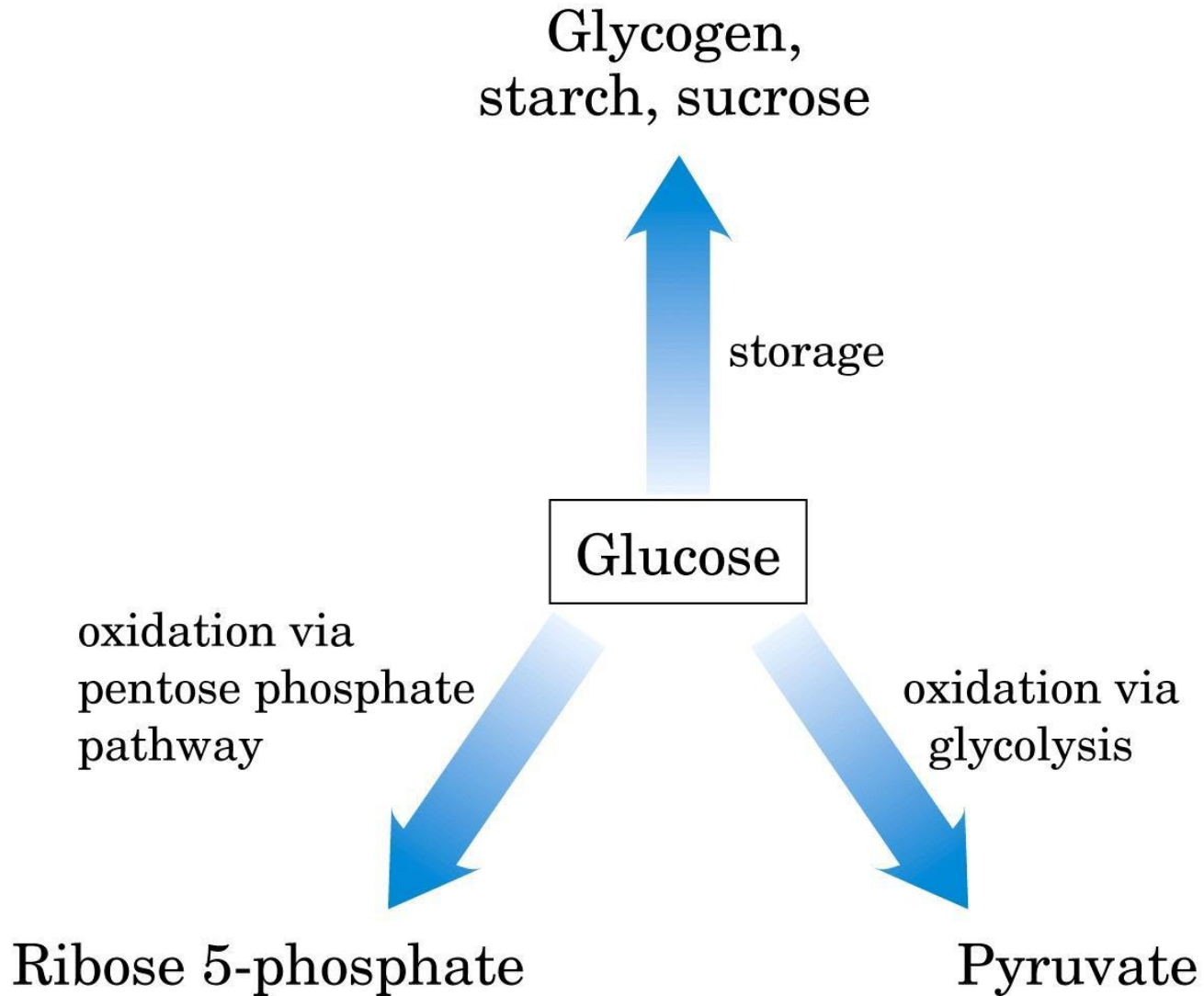
Dipartimento di Scienze Biomediche e  
Neuromotorie – DIBINEM – via Irnerio 48, Bologna

# GLYCOLYSIS (Embeden – Meyerhof – Parnas pathway)

- *The reactions of glycolysis*
- *Reoxidation of NADH from glycolysis*
- *Formation of lactic acid*
- *Shuttles*
- *Utilization as reducing power (biosynthesis)*
- *Energy balance*
- *Entry of other sugars into glycolysis*

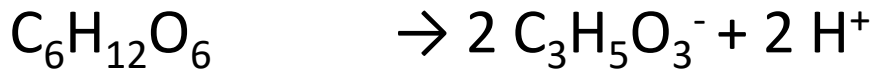


# GLYCOLYSIS (Embeden – Meyerhof – Parnas pathway)

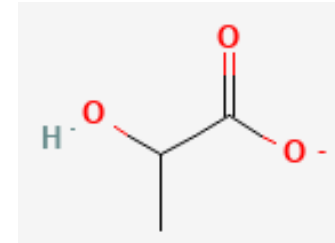
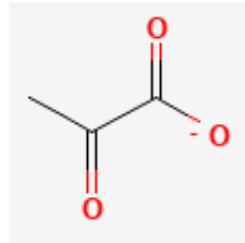
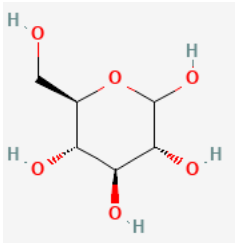
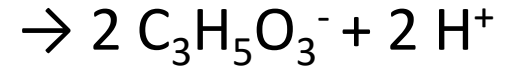
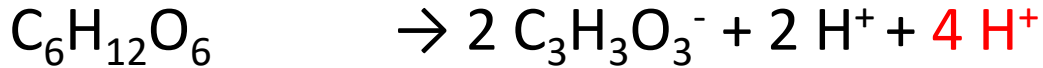


# GLYCOLYSIS (Embeden – Meyerhof – Parnas pathway)

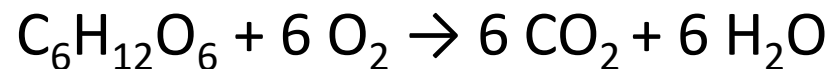
## Stoichiometry of glycolysis



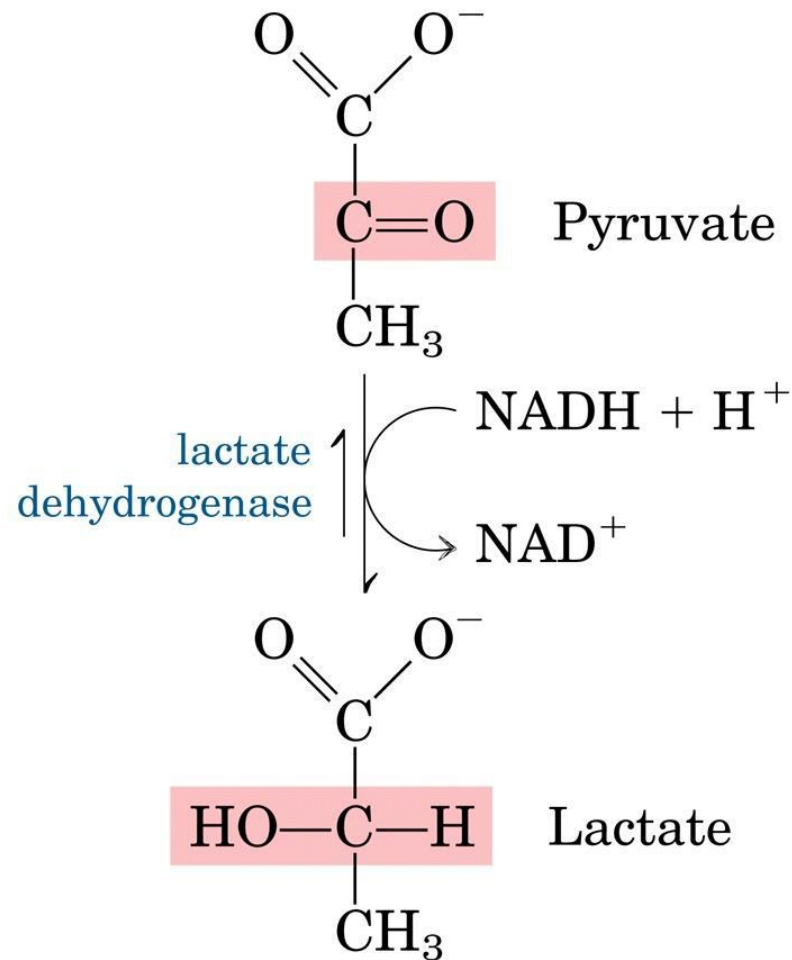
In reality:



Or, in aerobiosis:



# GLYCOLYSIS (Embeden – Meyerhof – Parnas pathway)



$$\Delta G'^{\circ} = -25.1 \text{ kJ/mol}$$



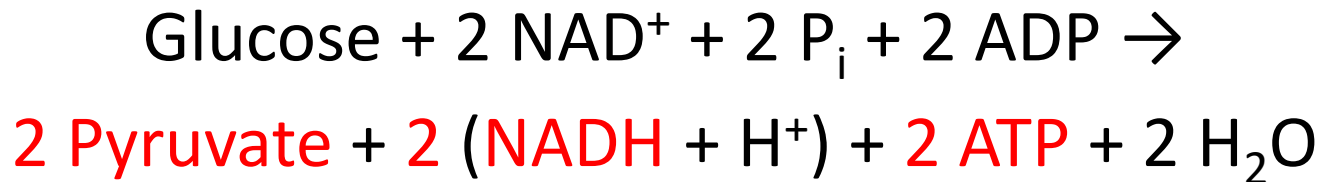
# GLYCOLYSIS (Embeden – Meyerhof – Parnas pathway)

## Net Reagents Participating in Glycolysis

Zymase (heat-labile)

Co-zymase (heat stable)

Required:  $P_i$ , ADP,  $NAD^+$ ,  $Mg^{2+}$  ...



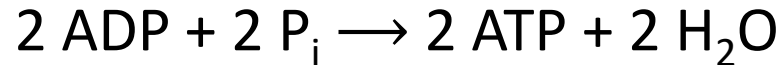
# GLYCOLYSIS (Embeden – Meyerhof – Parnas pathway)

The conversion of glucose to pyruvate is exergonic:



$$\Delta G'^{\circ}1 = -146 \text{ kJ/mol}$$

The formation of ATP from ADP and  $P_i$  is endergonic:



$$\Delta G'^{\circ}2 = 2 (30.5 \text{ kJ/mol}) = 61.0 \text{ kJ/mol}$$

The sum of the two processes gives the overall standard free-energy change of glycolysis,  $\Delta G'^{\circ} = \Delta G'^{\circ}1 + \Delta G'^{\circ}2 = -146 \text{ kJ/mol} + 61.0 \text{ kJ/mol} = -85 \text{ kJ/mol}$

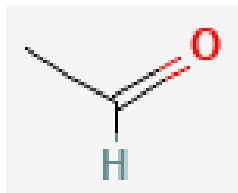
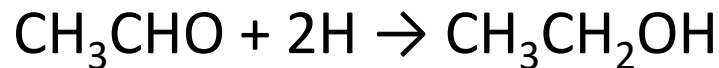
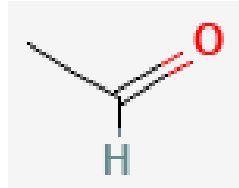
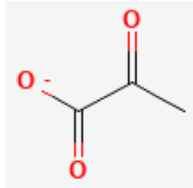
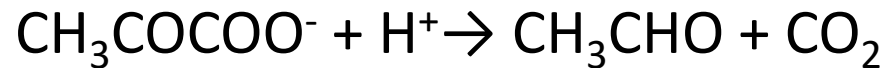
Under standard and cellular conditions, glycolysis is essentially irreversible.



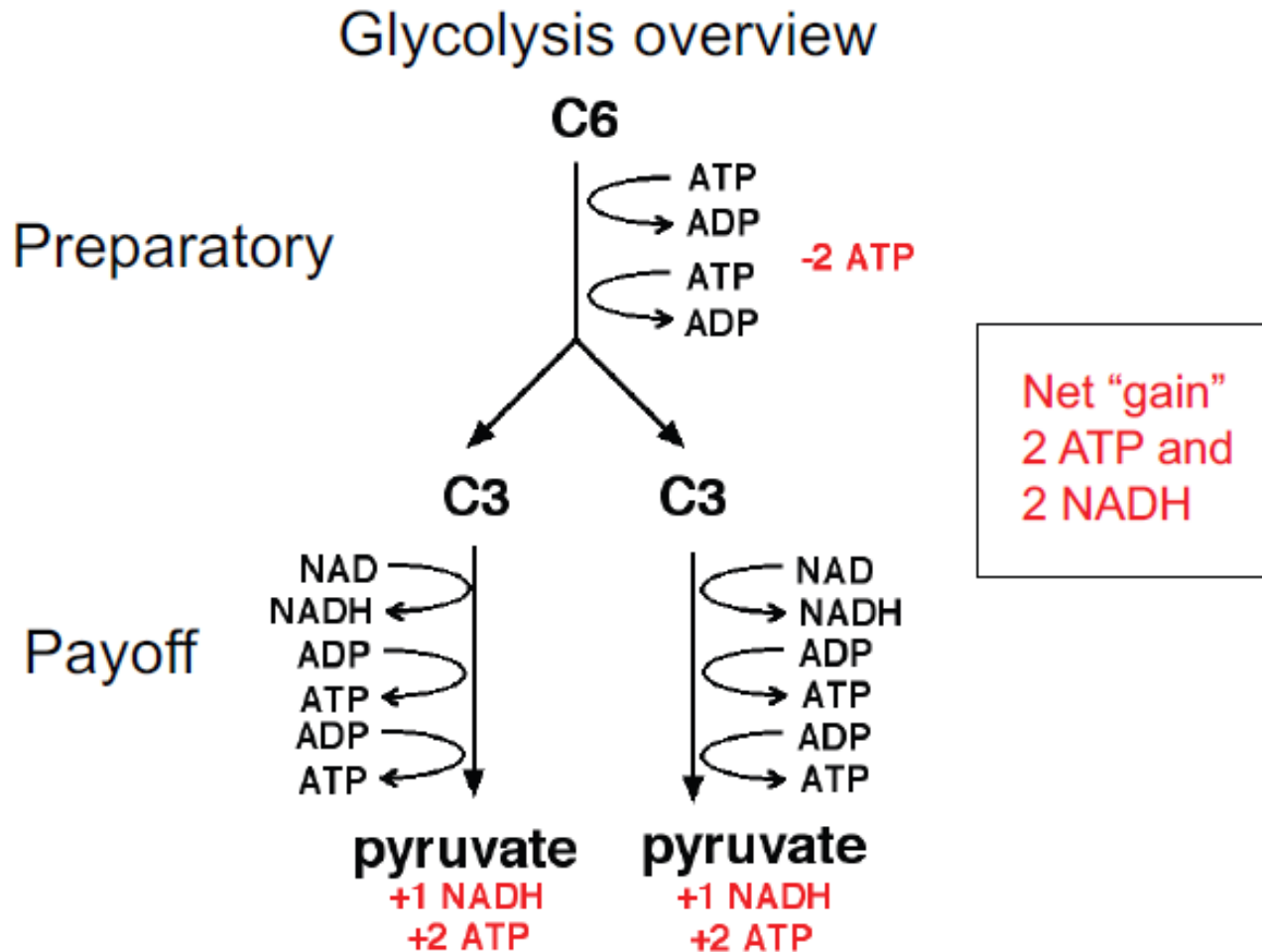
# GLYCOLYSIS (Embeden – Meyerhof – Parnas pathway)

Glycolysis was originally discovered by Pasteur as alcoholic fermentation in the yeast *Saccharomyces cerevisiae*.

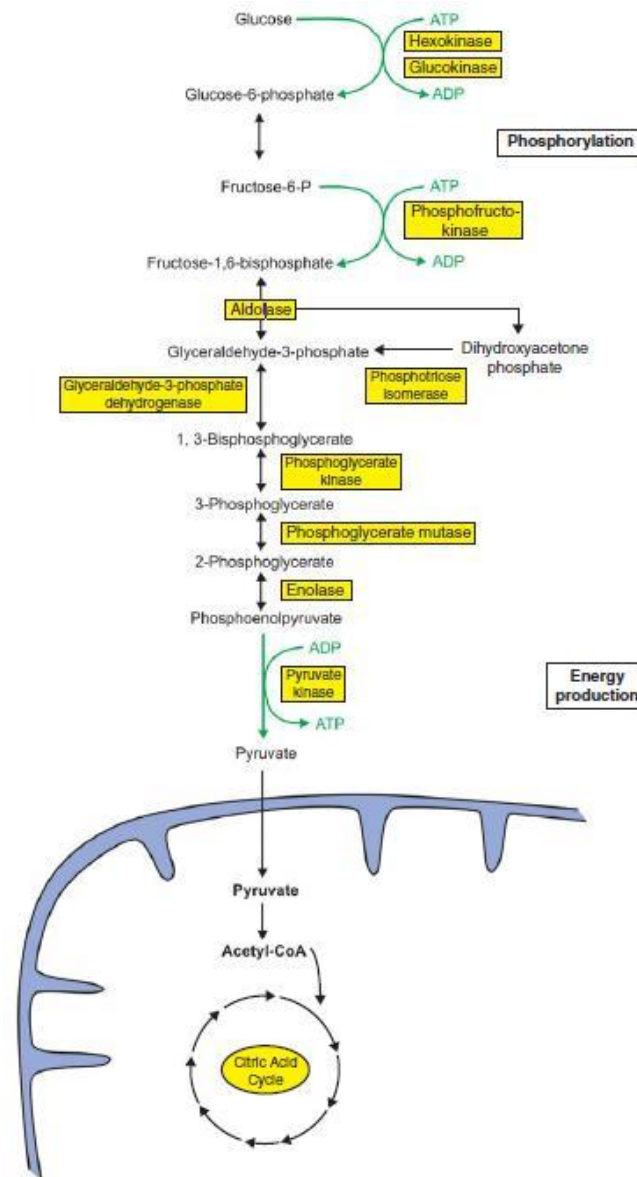
In yeast in anaerobiosis, pyruvate is decarboxylated to acetic aldehyde before reduction to ethanol:



# GLYCOLYSIS (Embeden – Meyerhof – Parnas pathway)



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# GLYCOLYSIS (Embeden – Meyerhof – Parnas pathway)

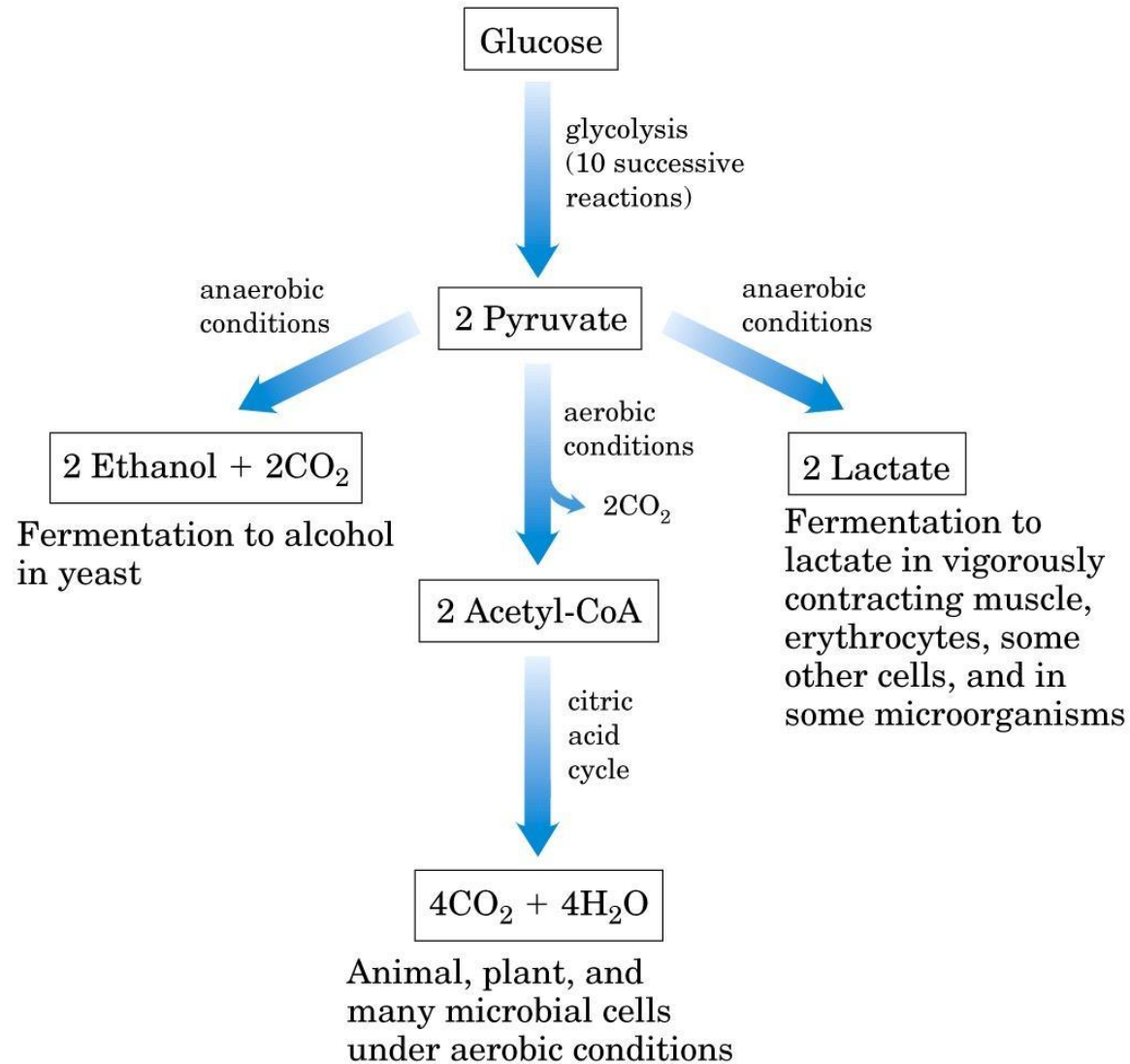
Energy stored in pyruvate can be extracted by:

- aerobic processes:
  - oxidative reactions in the citric acid cycle
  - oxidative phosphorylation
- anaerobic processes
  - reduction to lactate
  - reduction to ethanol

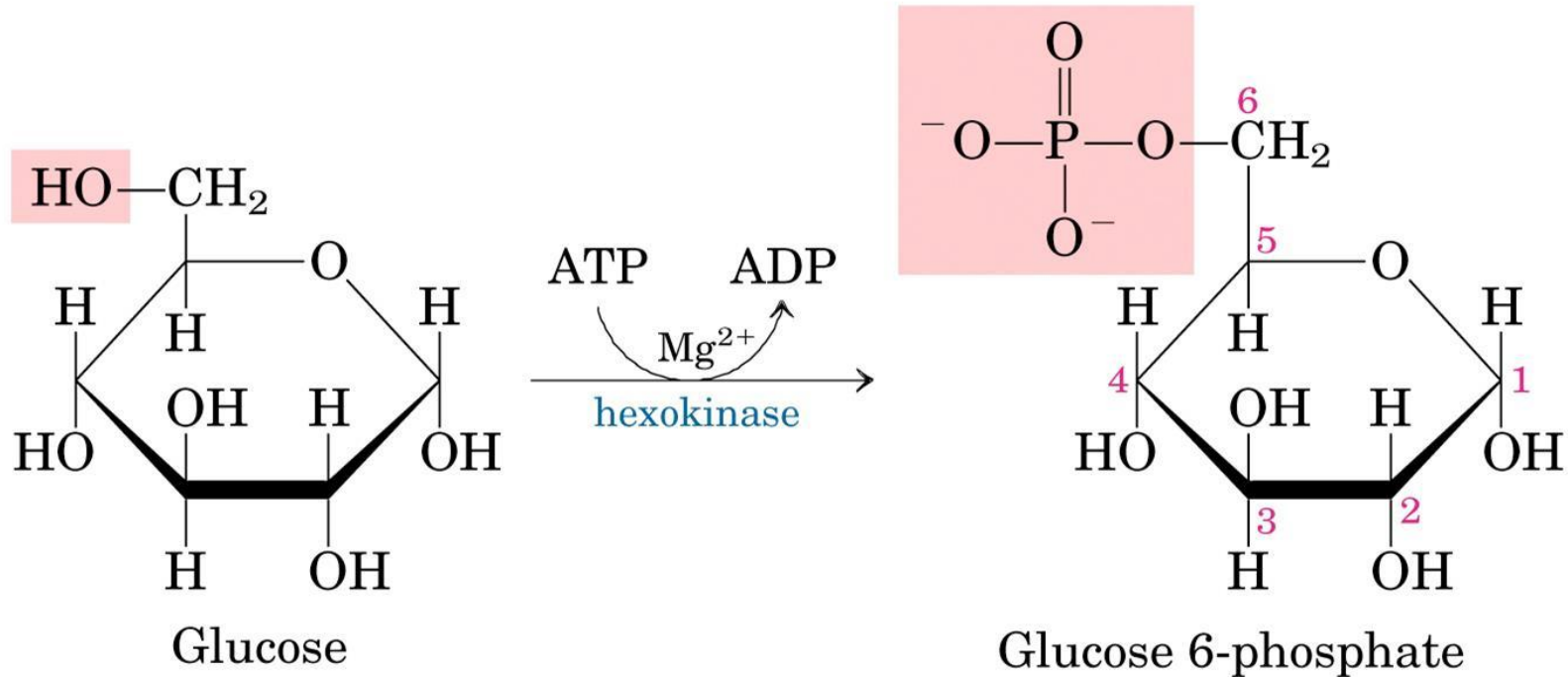
Pyruvate can provide the carbon skeleton for alanine synthesis or fatty acid synthesis.



# GLYCOLYSIS (Embeden – Meyerhof – Parnas pathway)



# GLYCOLYSIS (Embeden – Meyerhof – Parnas pathway)



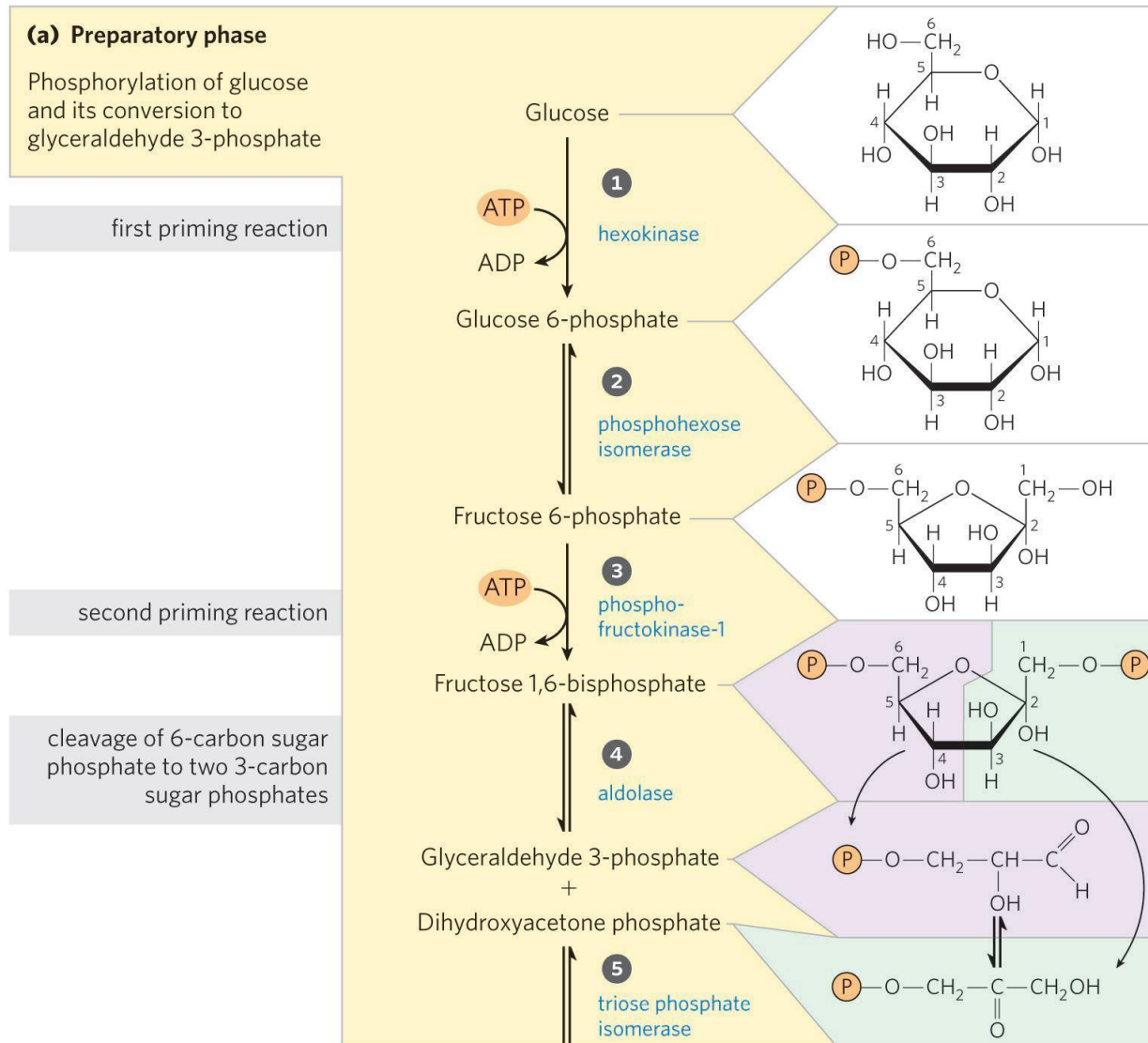
$$\Delta G'^{\circ} = -16.7 \text{ kJ/mol}$$

$$\Delta G = -16.7 + 6 \log \frac{[G6P] * [ADP]}{[Glu] * [ATP]}$$

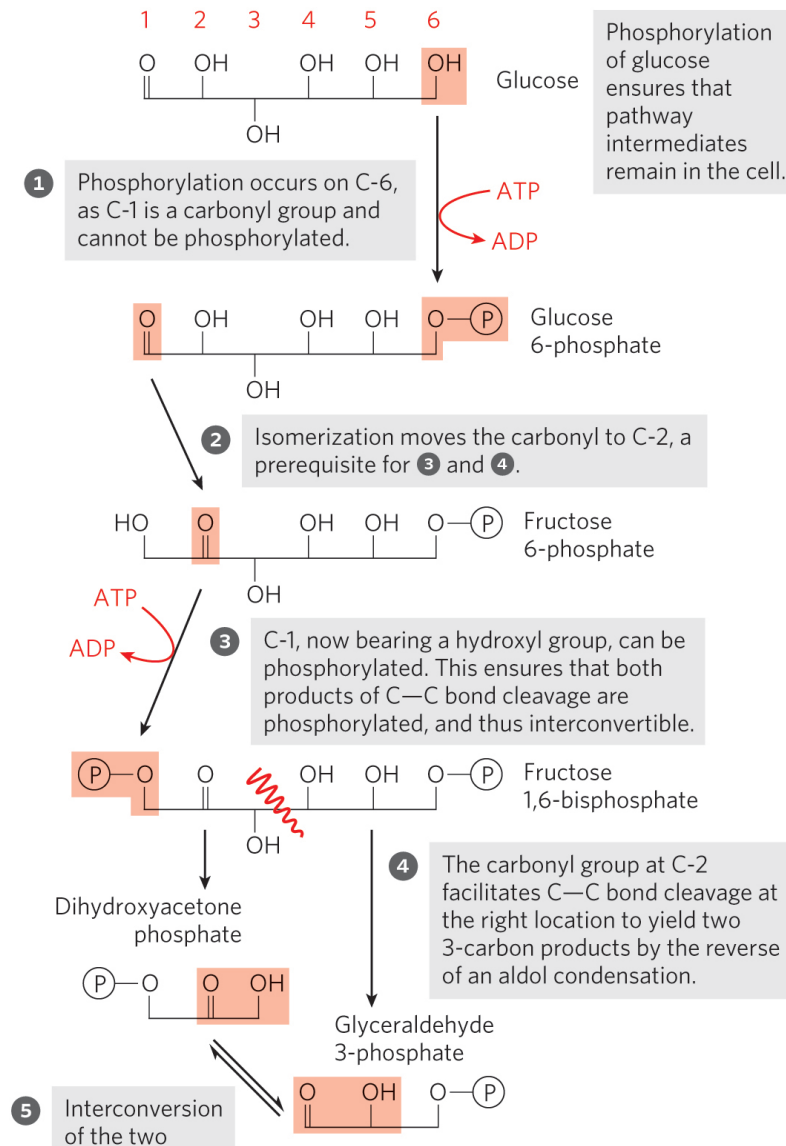
$[Glu] > [G6P]; [ATP] > [ADP] \rightarrow \Delta G$  even more negative



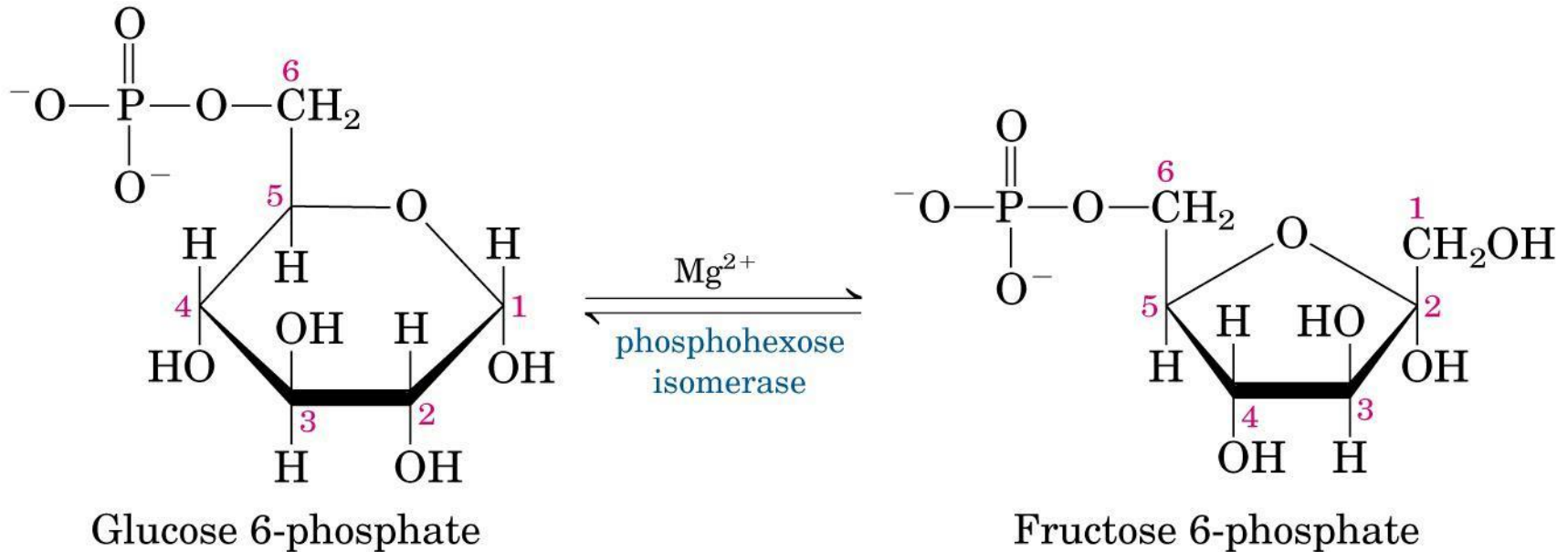
# GLYCOLYSIS (Embeden – Meyerhof – Parnas pathway)



# GLYCOLYSIS (Embeden – Meyerhof – Parnas pathway)

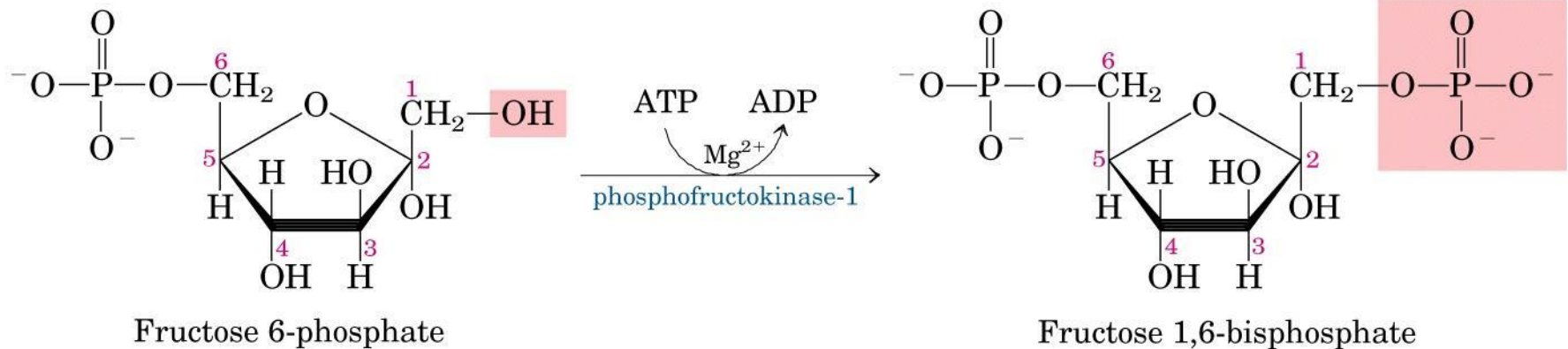


# GLYCOLYSIS (Embeden – Meyerhof – Parnas pathway)



$$\Delta G'^{\circ} = 1.7 \text{ kJ/mol}$$

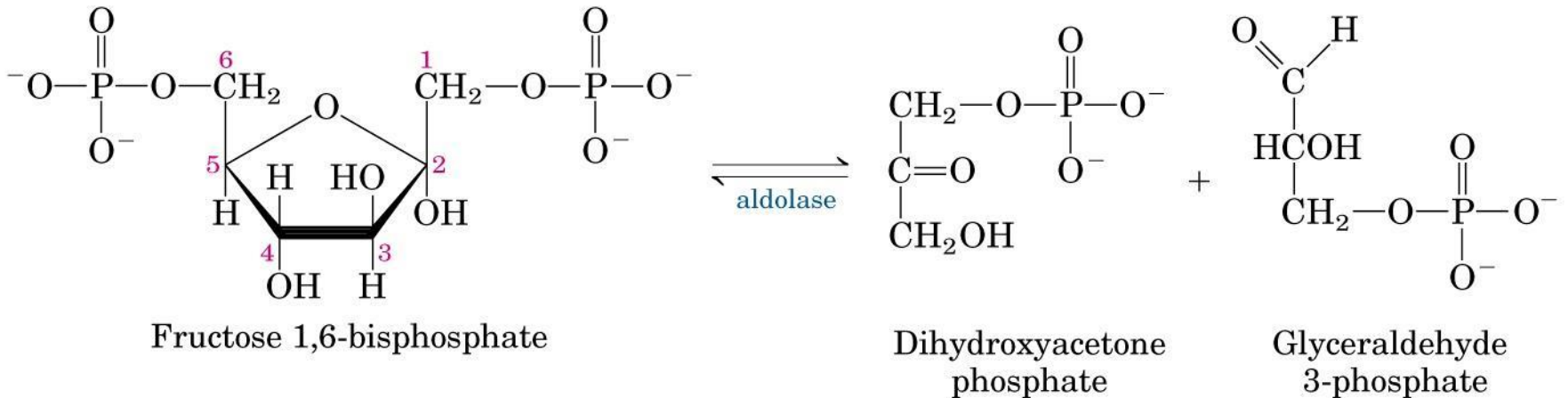
# GLYCOLYSIS (Embeden – Meyerhof – Parnas pathway)



$$\Delta G'^{\circ} = -14.2 \text{ kJ/mol}$$

Actual  $\Delta G$  much more negative.

# GLYCOLYSIS (Embeden – Meyerhof – Parnas pathway)



$$\Delta G'^{\circ} = 23.8 \text{ kJ/mol}$$

What is the  $\Delta G$  at the following metabolite concentration?

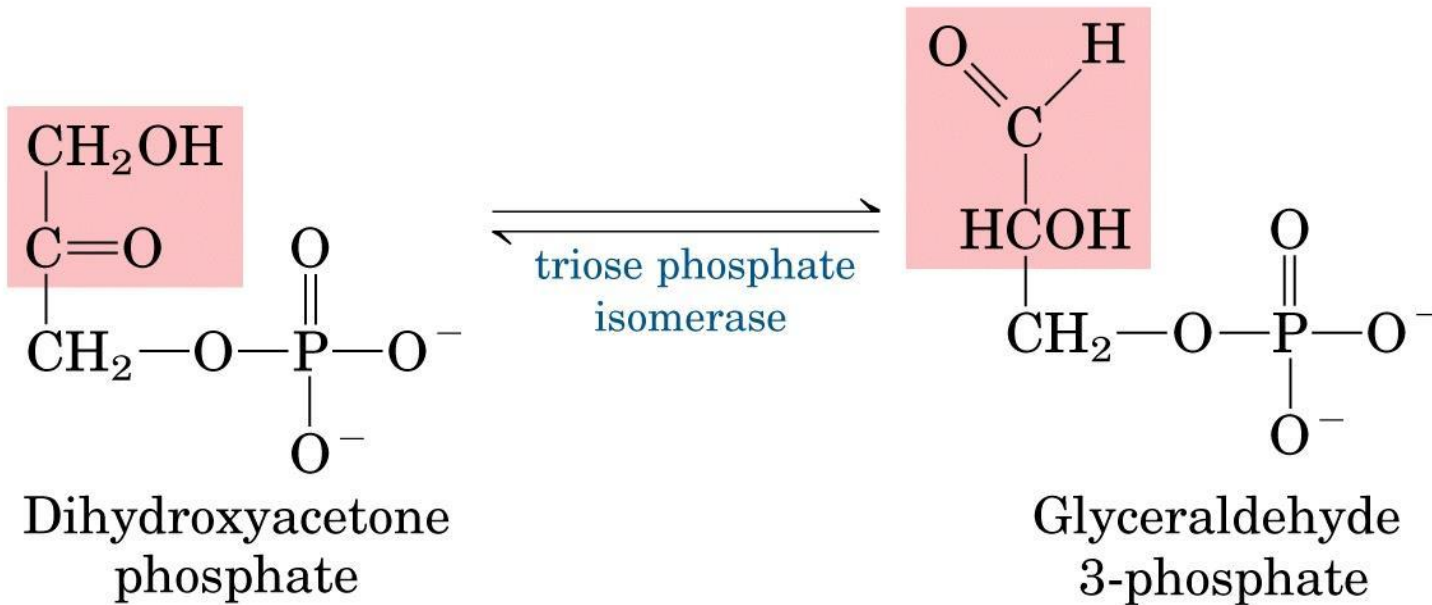
[FBP] = 10 mM; [GAP] = 1 mM; [DOP] = 1 mM

$$\Delta G = 24 + 6 \log \frac{10^{-3} \cdot 10^{-3}}{10^{-2}} = 24 + 6 \log 10^{-4} = 24 + 6(-4) = 0$$

Thus, depending on small concentration changes, the reaction can proceed in both directions (glycolysis or gluconeogenesis).



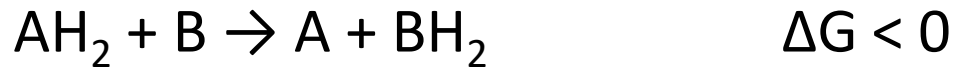
# GLYCOLYSIS (Embeden – Meyerhof – Parnas pathway)



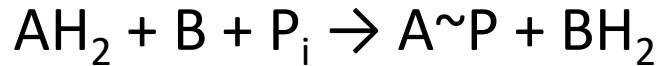
$$\Delta G'^{\circ} = 7.5 \text{ kJ/mol}$$

# GLYCOLYSIS (Embeden – Meyerhof – Parnas pathway)

## Substrate level phosphorylation:

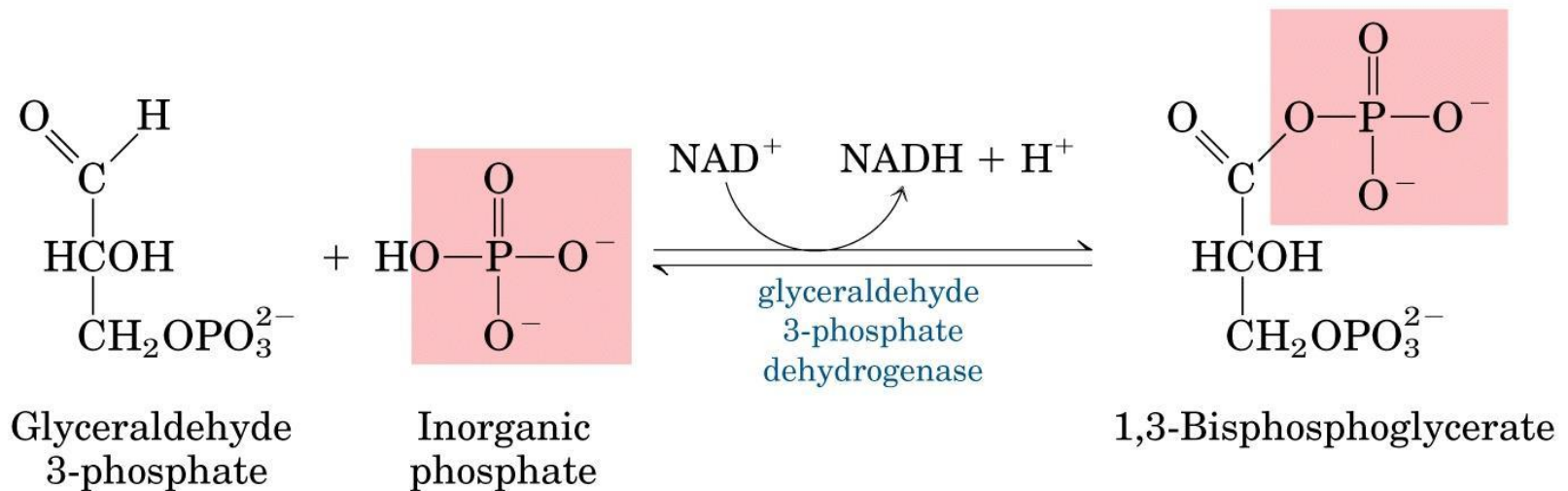


## Mechanism



# GLYCOLYSIS (Embeden – Meyerhof – Parnas pathway)

The strongly exergonic oxidation of an aldehyde to a carboxylic acid is exploited for the strongly endergonic incorporation of phosphate into the carboxyl group. The resulting reaction has a small free energy change and is reversible.



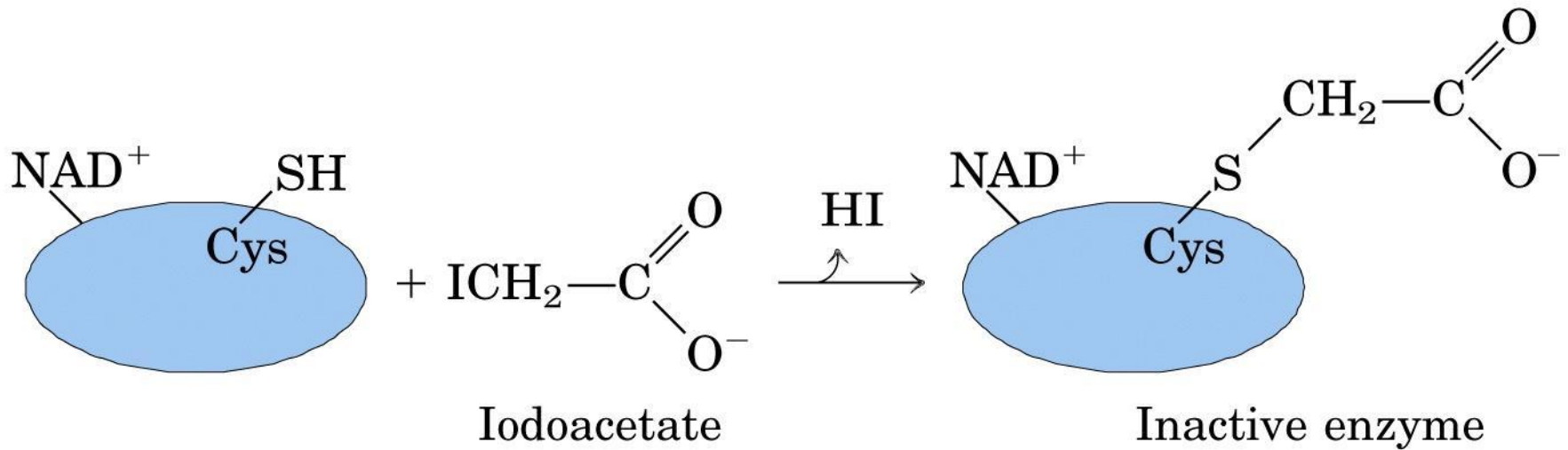
$$\Delta G'^{\circ} = 6.3 \text{ kJ/mol}$$





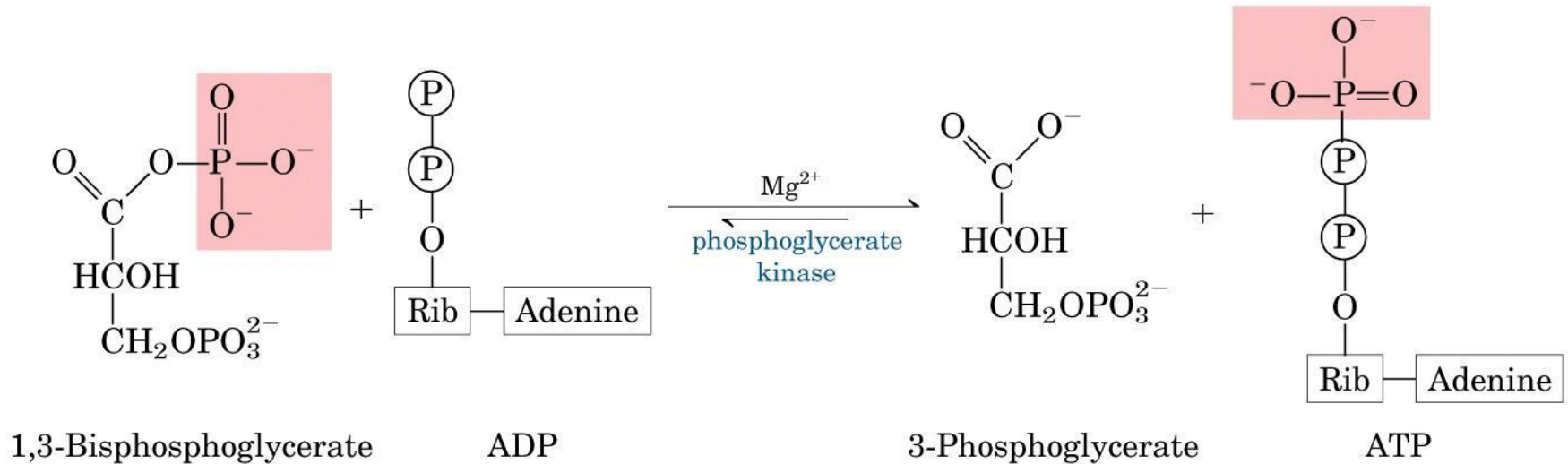
# GLYCOLYSIS (Embeden – Meyerhof – Parnas pathway)

Mechanism of glyceraldehyde 3-phosphate dehydrogenase



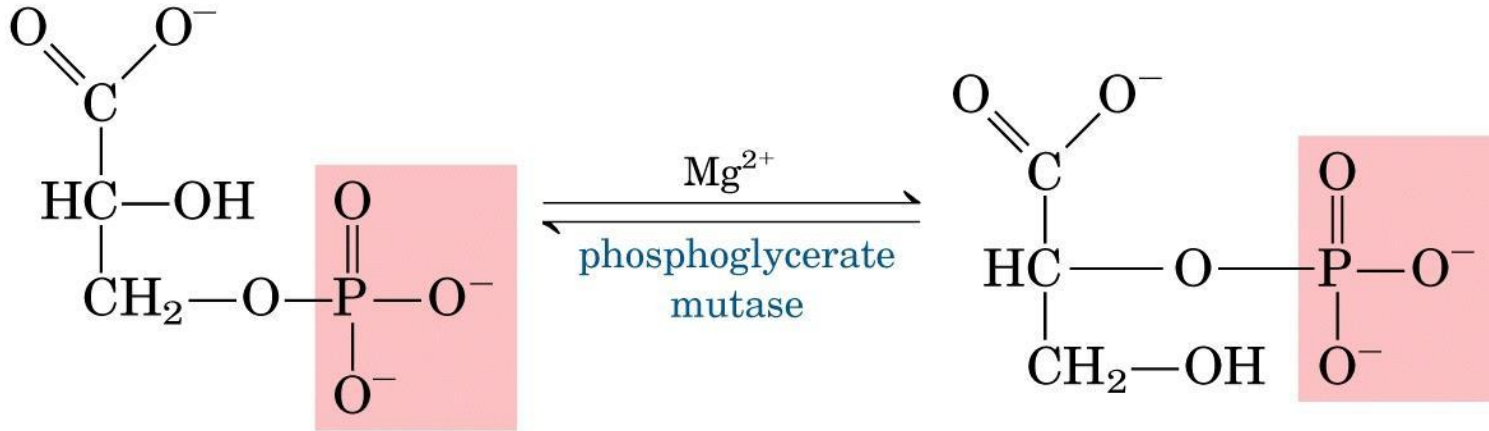
# GLYCOLYSIS (Embeden – Meyerhof – Parnas pathway)

Concentration of products is higher than that of reagents, therefore  $\Delta G$  is close to 0: reaction is reversible and also occurs in gluconeogenesis.



$$\Delta G'^{\circ} = -18.5 \text{ kJ/mol}$$

# GLYCOLYSIS (Embeden – Meyerhof – Parnas pathway)

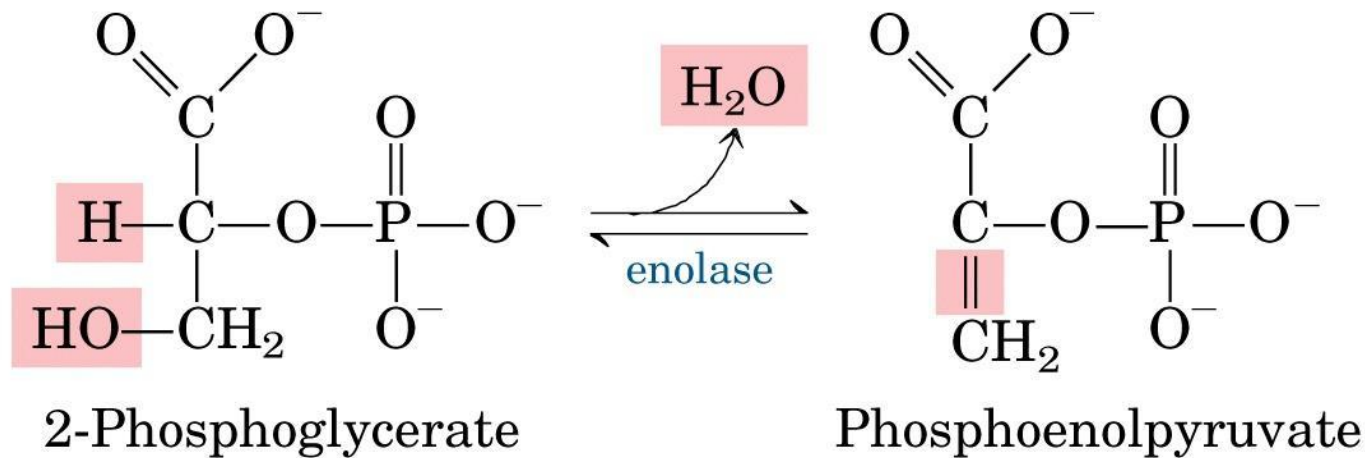


3-Phosphoglycerate

2-Phosphoglycerate

$$\Delta G'^{\circ} = 4.4 \text{ kJ/mol}$$

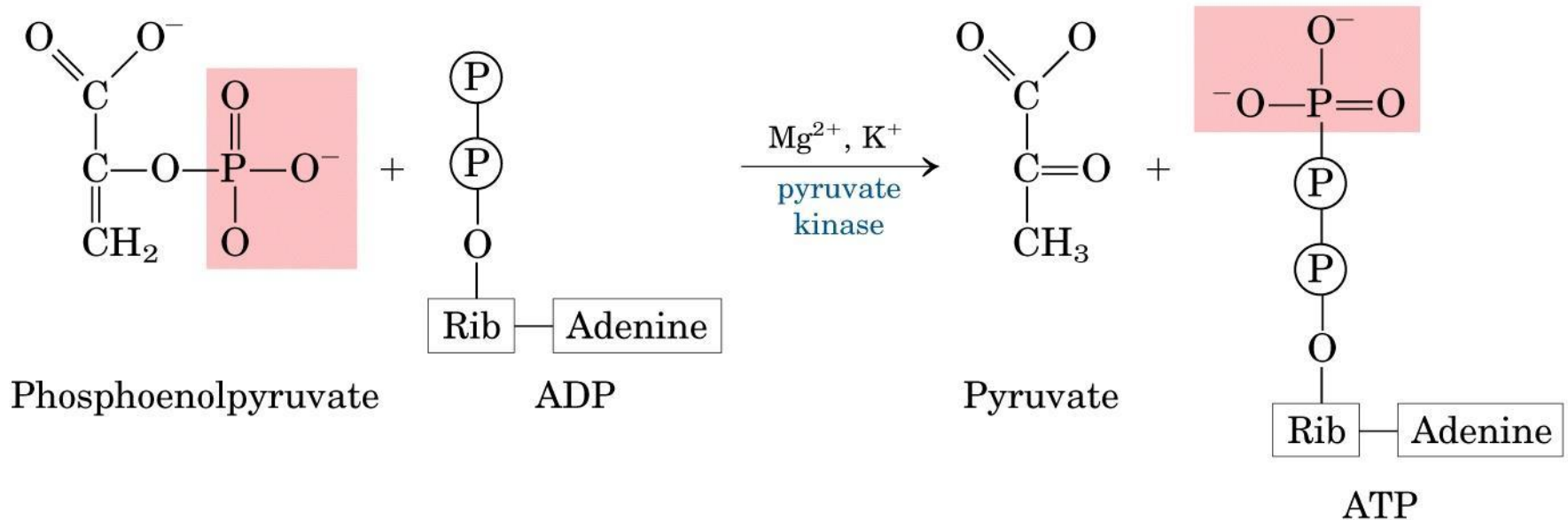
# GLYCOLYSIS (Embeden – Meyerhof – Parnas pathway)



$$\Delta G'^{\circ} = 7.5 \text{ kJ/mol}$$

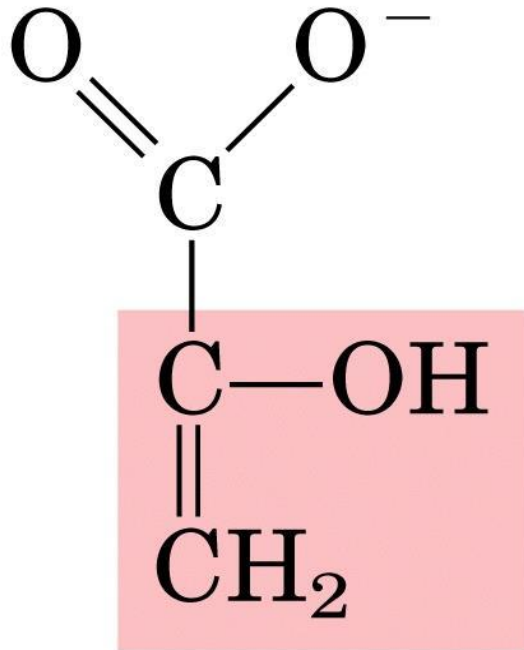
# GLYCOLYSIS (Embeden – Meyerhof – Parnas pathway)

Since  $[ATP] > [ADP]$ ,  $\Delta G > \Delta G^\circ$ , but still negative enough to make the reaction irreversible.

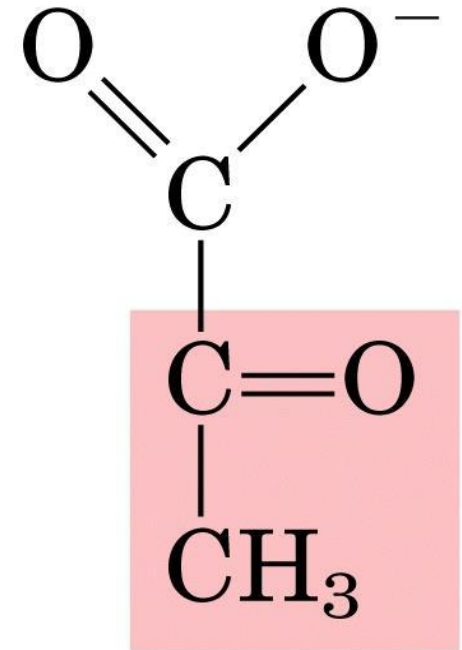


$$\Delta G'^{\circ} = -31.4 \text{ kJ/mol}$$

# GLYCOLYSIS (Embeden – Meyerhof – Parnas pathway)



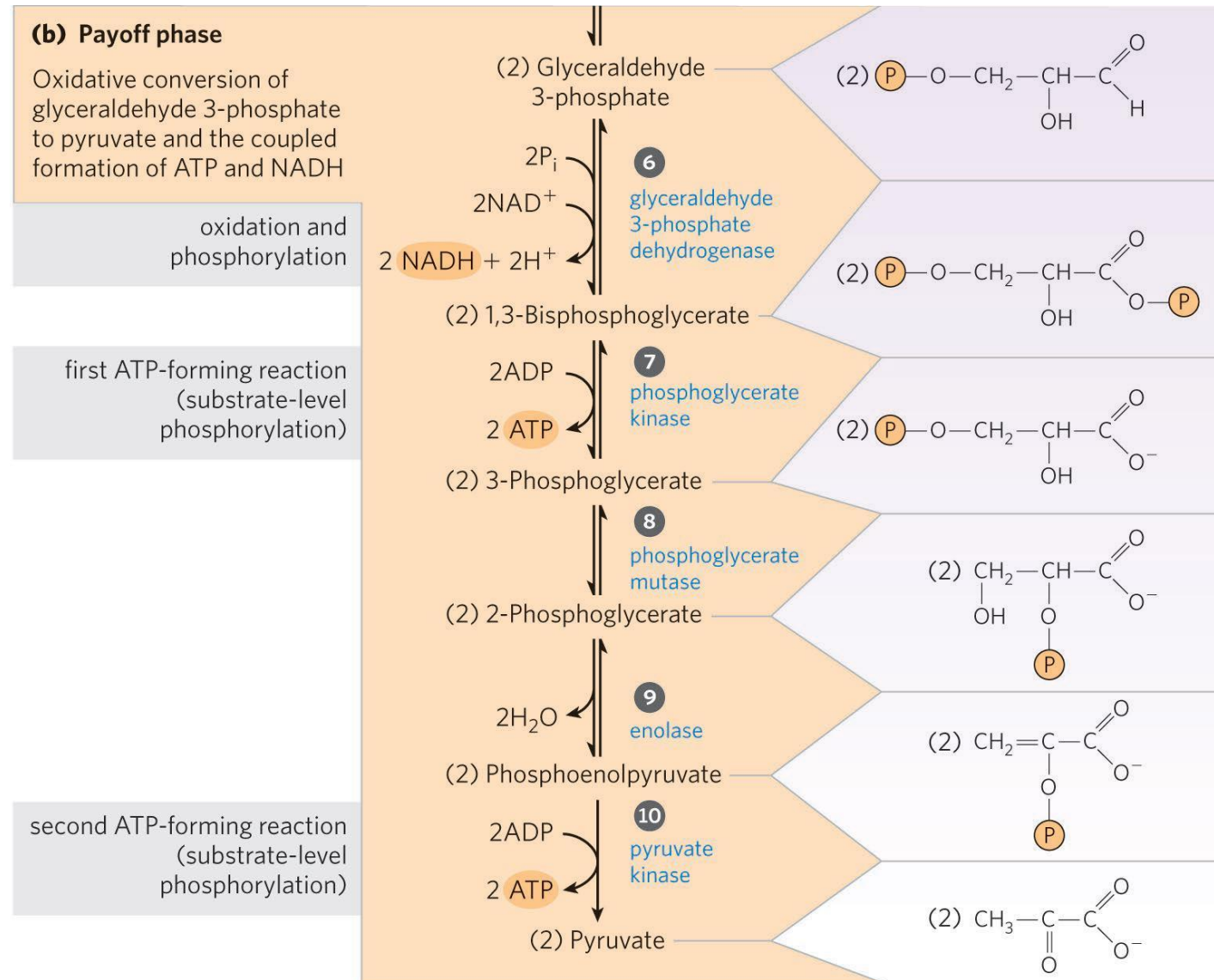
Pyruvate  
(enol form)



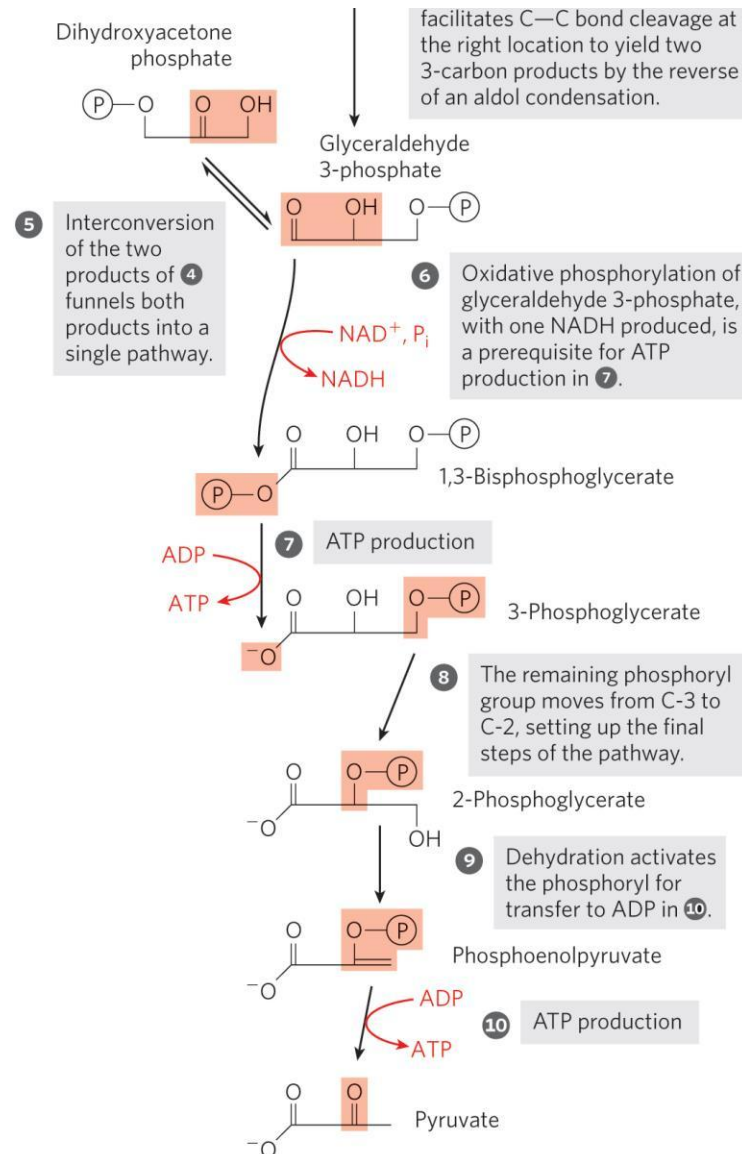
Pyruvate  
(keto form)



# GLYCOLYSIS (Embeden – Meyerhof – Parnas pathway)



# GLYCOLYSIS (Embeden – Meyerhof – Parnas pathway)



# GLYCOLYSIS (Embeden – Meyerhof – Parnas pathway)

table 20-1

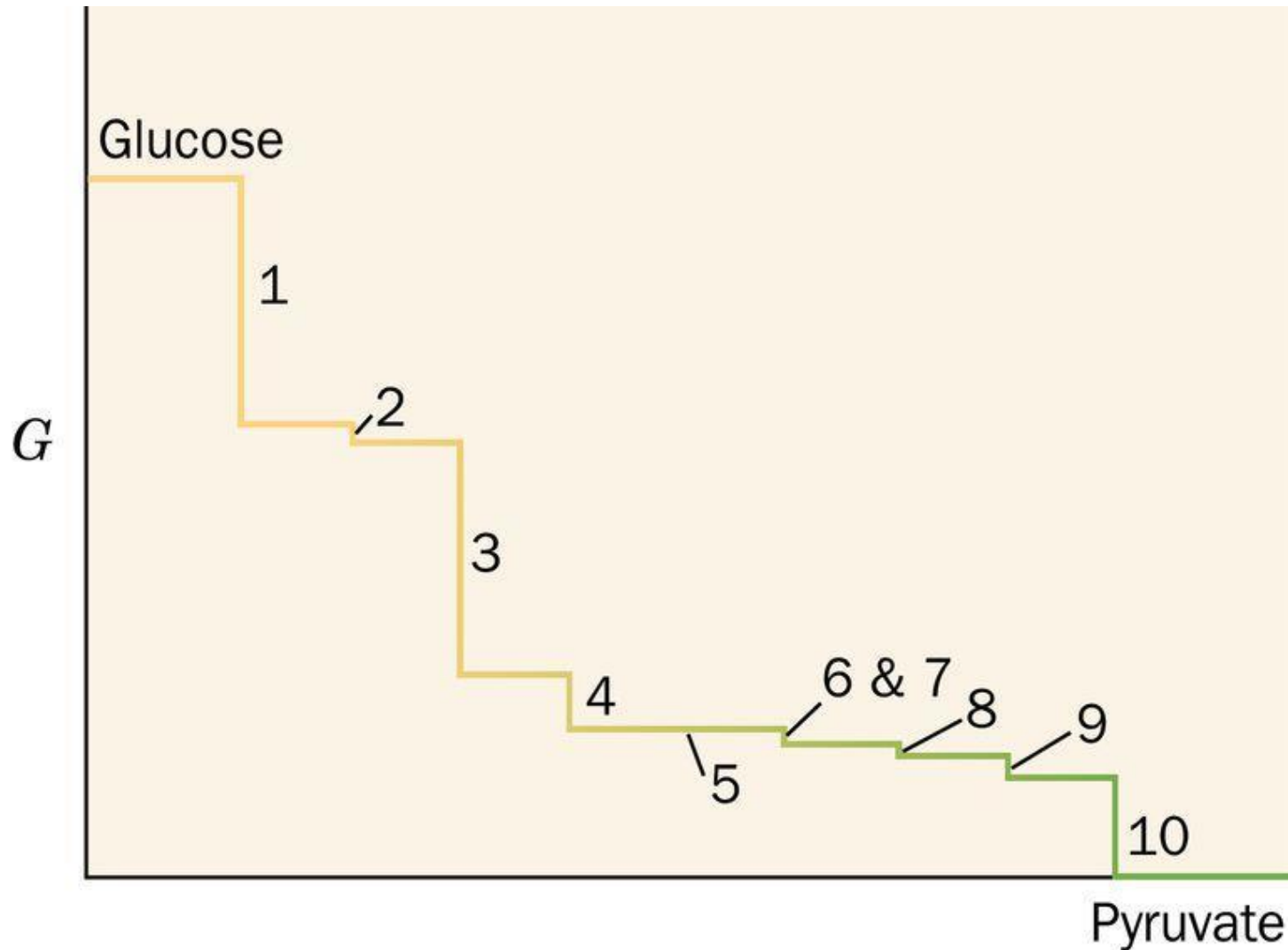
Free-Energy Changes of Glycolytic Reactions in Erythrocytes\*

Glycolytic reaction step	$\Delta G'^{\circ}$ (kJ/mol)	$\Delta G$ (kJ/mol)
① Glucose + ATP $\longrightarrow$ glucose 6-phosphate + ADP + H <sup>+</sup>	-16.7	-33.4
② Glucose 6-phosphate $\rightleftharpoons$ fructose 6-phosphate	1.7	-2.5
③ Fructose 6-phosphate + ATP $\longrightarrow$ fructose 1,6-bisphosphate + ADP + H <sup>+</sup>	-14.2	-22.2
④ Fructose 1,6-bisphosphate $\rightleftharpoons$ dihydroxyacetone phosphate + glyceraldehyde 3-phosphate	23.8	-1.25
⑤ Dihydroxyacetone phosphate $\rightleftharpoons$ glyceraldehyde 3-phosphate	7.5	2.5
⑥ Glyceraldehyde 3-phosphate + P <sub>i</sub> + NAD <sup>+</sup> $\rightleftharpoons$ 1,3-bisphosphoglycerate + NADH + H <sup>+</sup>	6.3	-1.7
⑦ 1,3-Bisphosphoglycerate + ADP $\rightleftharpoons$ 3-phosphoglycerate + ATP	-18.8	1.25
⑧ 3-Phosphoglycerate $\rightleftharpoons$ 2-phosphoglycerate	4.4	0.8
⑨ 2-Phosphoglycerate $\rightleftharpoons$ phosphoenolpyruvate + H <sub>2</sub> O	7.5	-3.3
⑩ Phosphoenolpyruvate + ADP + H <sup>+</sup> $\longrightarrow$ pyruvate + ATP	-31.4	-16.7

\* $\Delta G'^{\circ}$  is the standard free-energy change, as defined in Chapter 14 (see p. 494). At pH 7.0,  $\Delta G$  is the free-energy change calculated from the actual concentrations of glycolytic intermediates present under physiological conditions in erythrocytes. The glycolytic reactions bypassed in gluconeogenesis are shown in red.

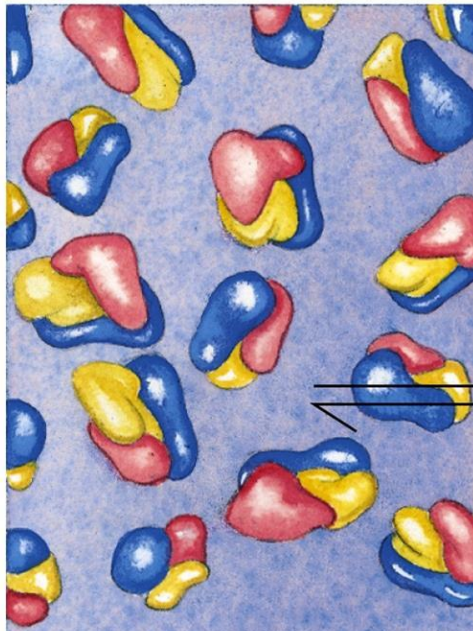


# GLYCOLYSIS (Embeden – Meyerhof – Parnas pathway)

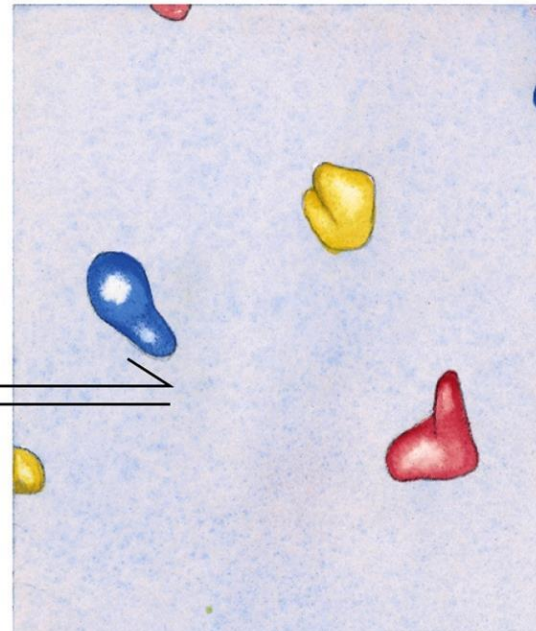


# GLYCOLYSIS (Embeden – Meyerhof – Parnas pathway)

In the cell, the enzymes of glycolysis are assembled into multi-enzyme complexes *in vivo*.

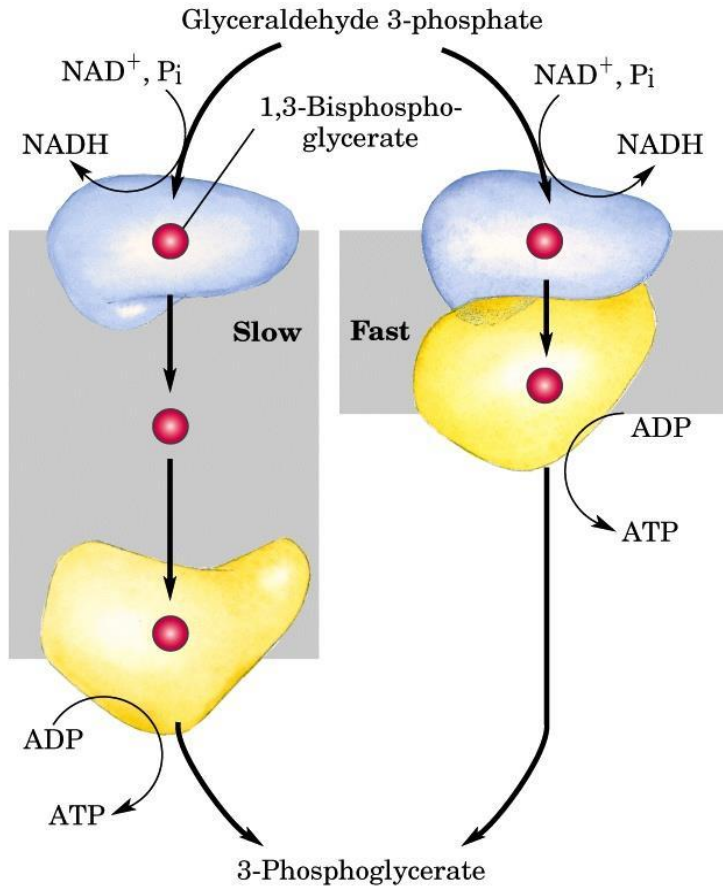


In the cytosol, high concentrations of enzymes 1, 2, and 3 favor their association.



In extract of broken cells, dilution by buffer reduces the concentrations of enzymes 1, 2, and 3, favoring their dissociation.

# GLYCOLYSIS (Embeden – Meyerhof – Parnas pathway)



glyceraldehyde-3-phosphate dehydrogenase (GAPDH)

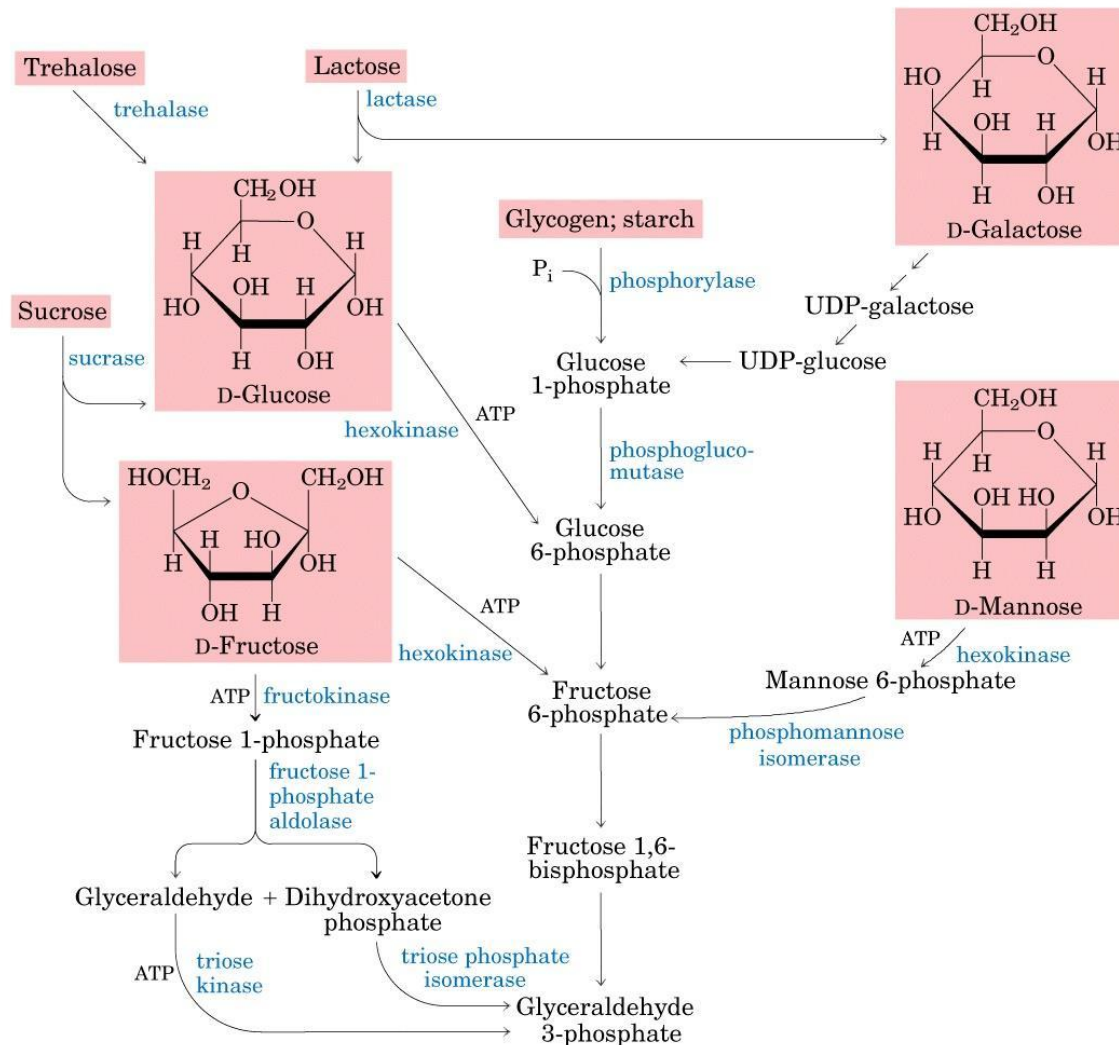
Phosphoglycerate kinase

Sequential action of two separate enzymes: the product of the first enzyme (1,3-bisphosphoglycerate) diffuses to the second enzyme.

Substrate channeling through a functional complex of two enzymes: the intermediate (1,3-bisphosphoglycerate) is never released to the solvent.

# GLYCOLYSIS (Embeden – Meyerhof – Parnas pathway)

## Other carbohydrates entering glycolysis



# GLYCOLYSIS (Embeden – Meyerhof – Parnas pathway)

**Liver fructokinase** has very high activity and is not regulated (contrary to PFK). Excessive glycolytic flux leads to increased acetyl-CoA and fatty acid synthesis, while high dioxyacetone-P is converted to glycerol-P.

Fatty acids and glycerol-P enhance **lipid synthesis**, contributing to fatty liver, obesity, and metabolic syndrome.



# REGULATION OF GLYCOLYSIS IN MUSCLES

The 3 irreversible steps are catalyzed by allosteric enzymes:

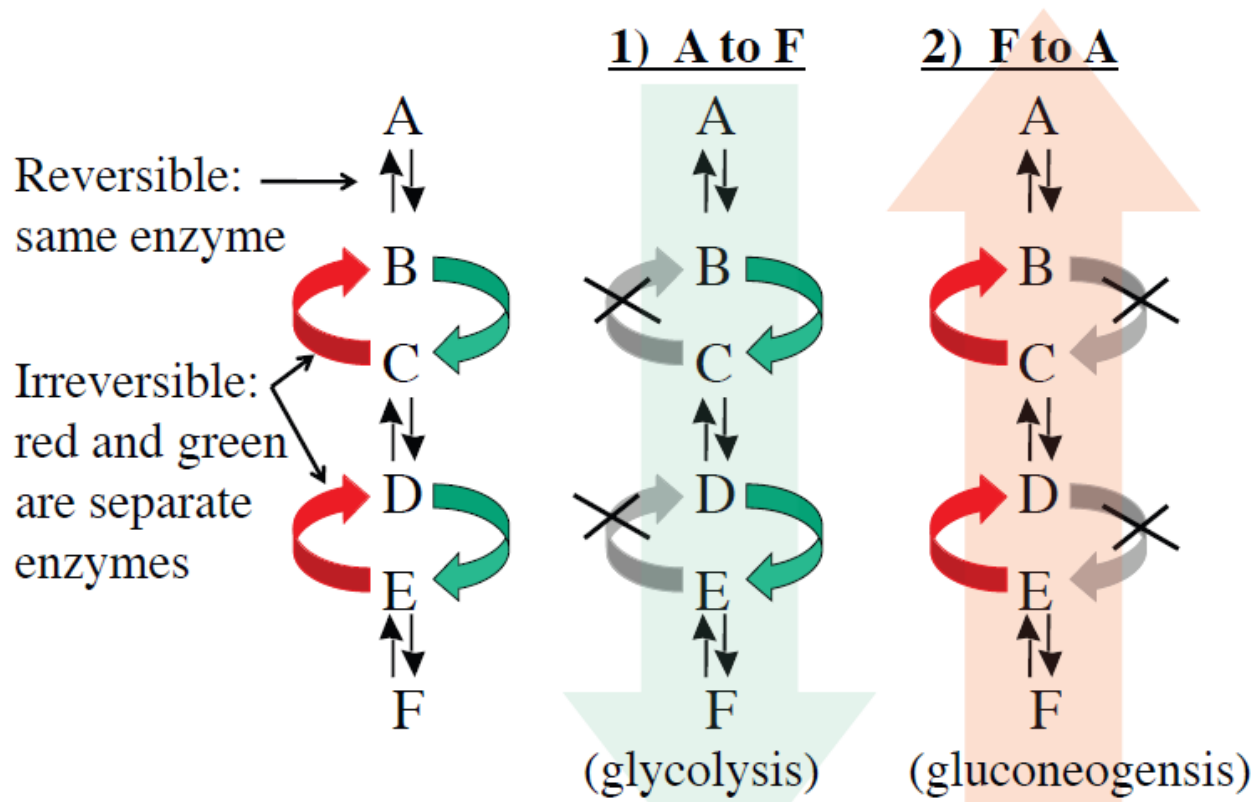
- **Hexokinase** (inhibited by glucose-6-P)
- **Phosphofructokinase** (inhibited by ATP, citrate; activated by AMP, fructose-2,6-bisP)
- **Pyruvate kinase** (activated by fructose-1,6-bisP, inhibited by acetyl-CoA)



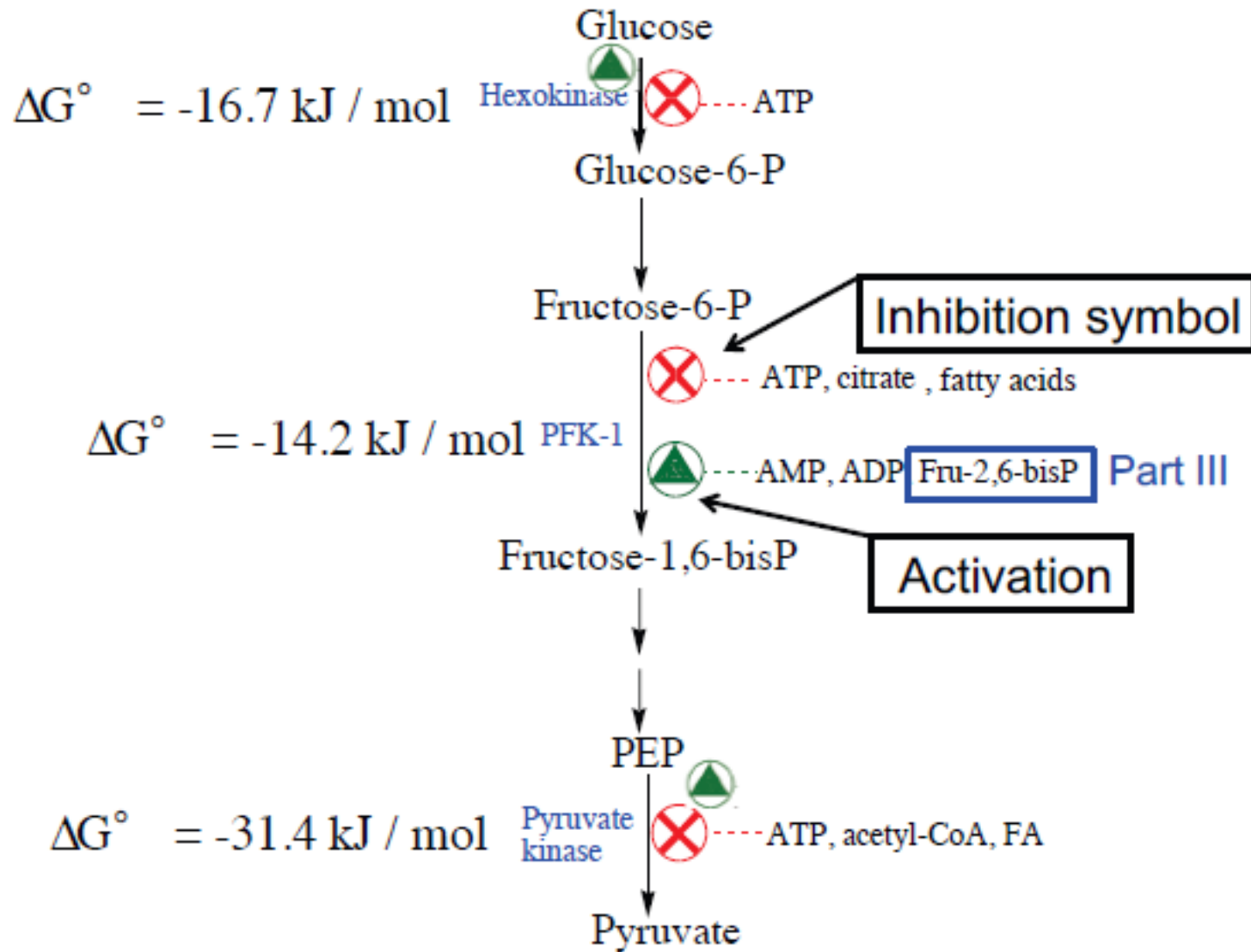
# REGULATION OF GLYCOLYSIS IN LIVER

In liver, regulation of glycolysis is strictly linked and symmetrical with regulation of gluconeogenesis.

One-way regulation at “irreversible” steps  
(depending upon “needs” of cell)

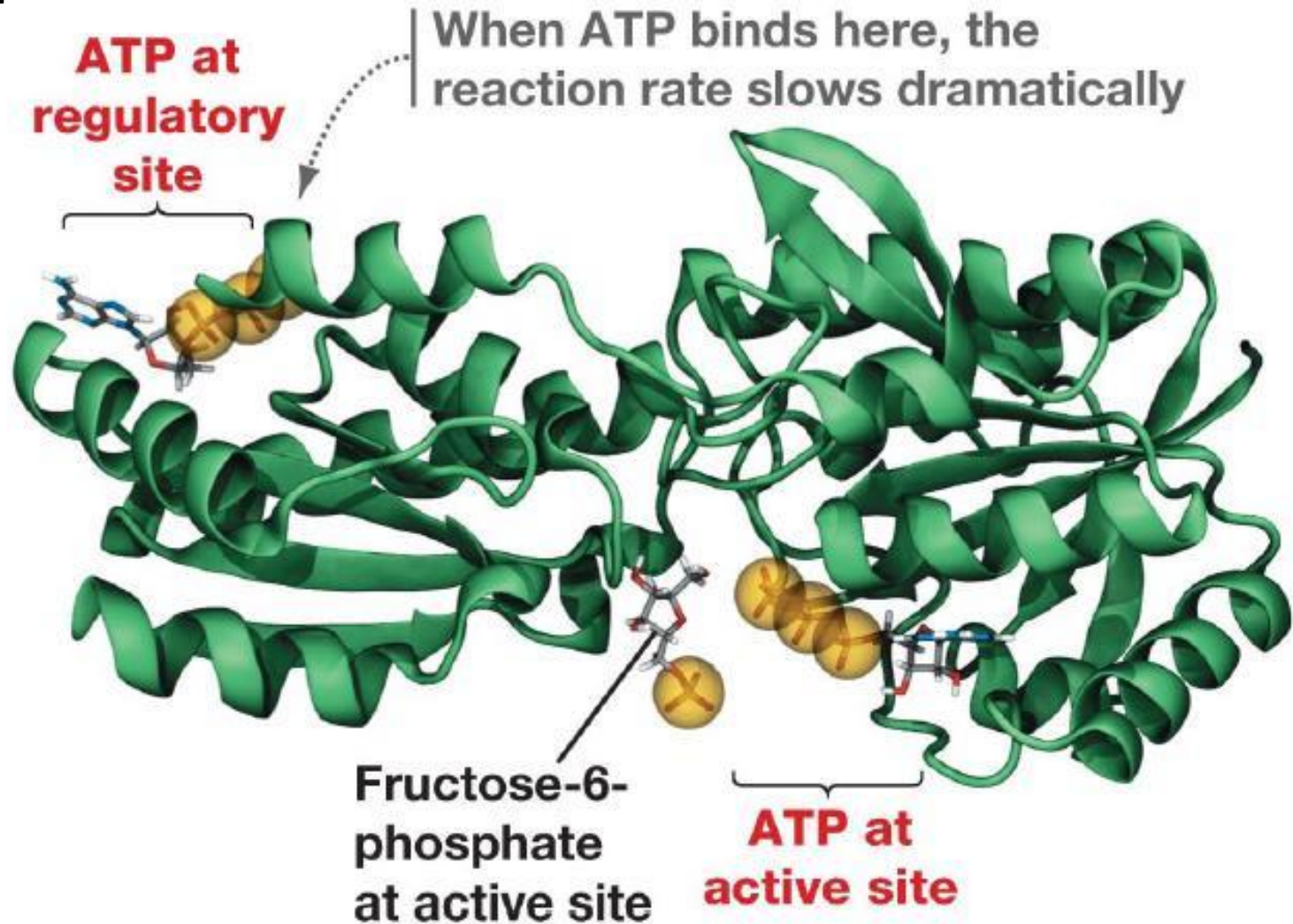


# REGULATION OF GLYCOLYSIS IN MUSCLES



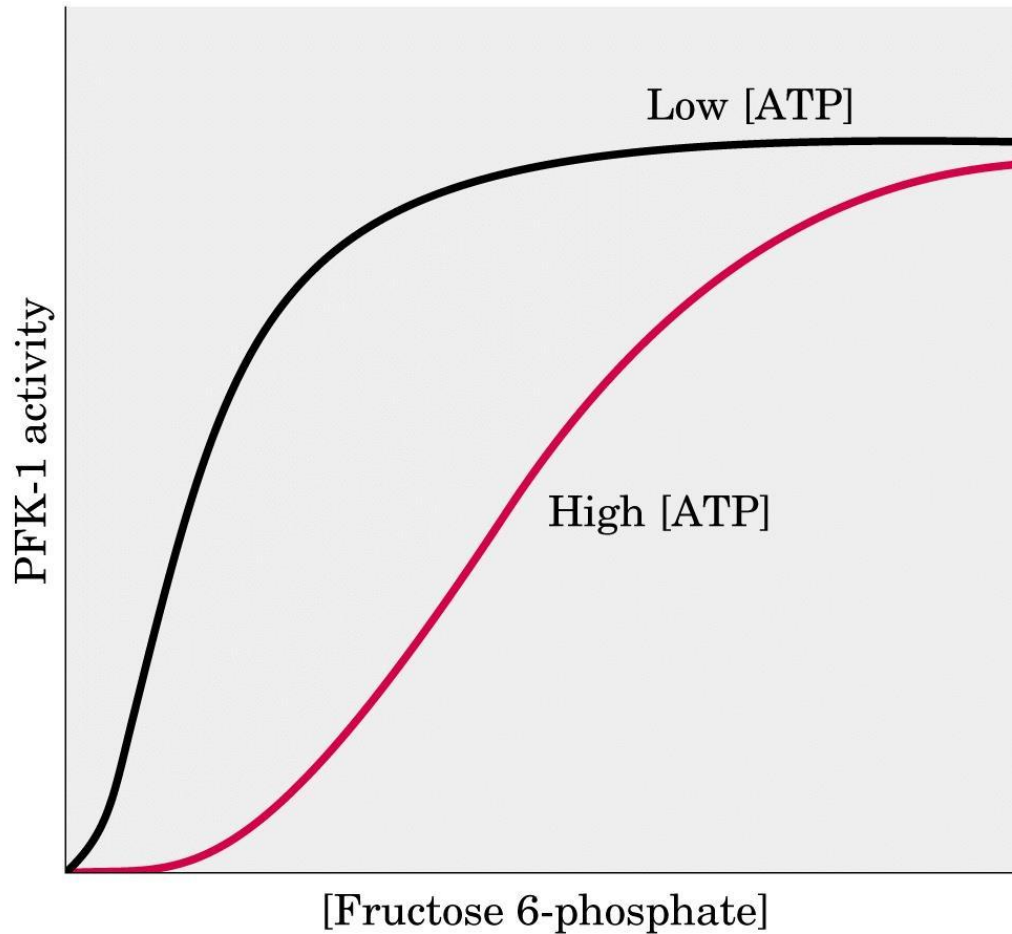
# REGULATION OF GLYCOLYSIS

## Phosphofructokinase



# REGULATION OF GLYCOLYSIS

## Phosphofructokinase



(b)

# REGULATION OF GLYCOLYSIS

Why are intermediates phosphorylated?

- Prevent glycolytic intermediates from leaving the cell.
- Because they allow the three **irreversible control steps**.

Let's figure out a glycolysis without the initial phosphorylation steps:

Glucose → fructose → (glyceraldehyde + dioxyacetone) → 1-P-glycerate → glycerate → pyruvate

The irreversible control steps are lost!!!!



**Credits:**

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