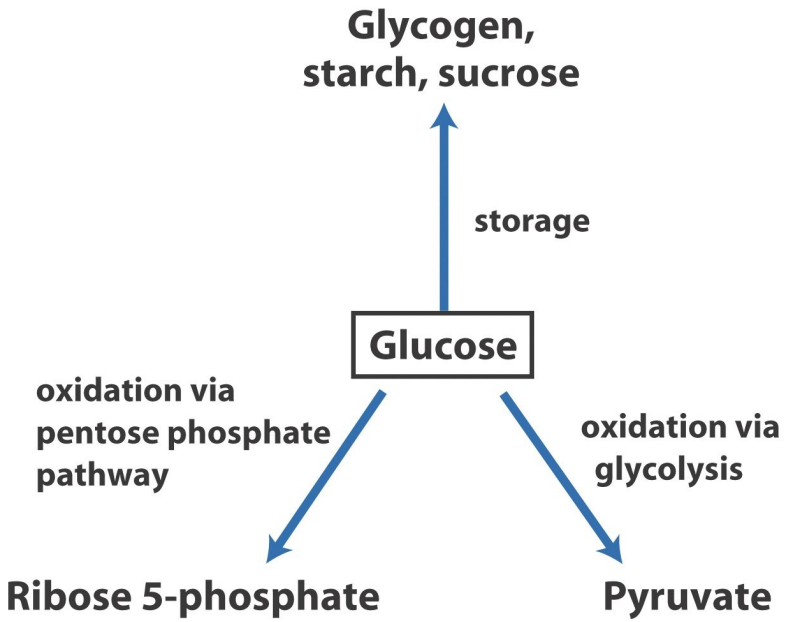
Department of Chemistry and Biochemistry
University of Lethbridge

University of Lethbridge

III. Metabolism
- Glycolysis

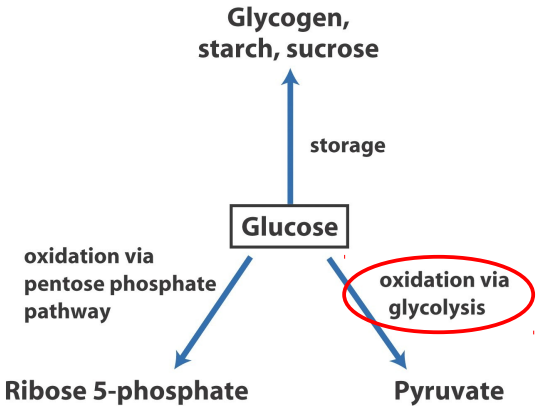
Major Pathways of Glucose Utilization



These three pathways are the most significant in terms of the amount of glucose that flows through them in most cells.

The Two Phases of Glycolysis

Breakdown of the glucose (6C) into two molecules of the pyruvate (3C) occurs in ten steps.

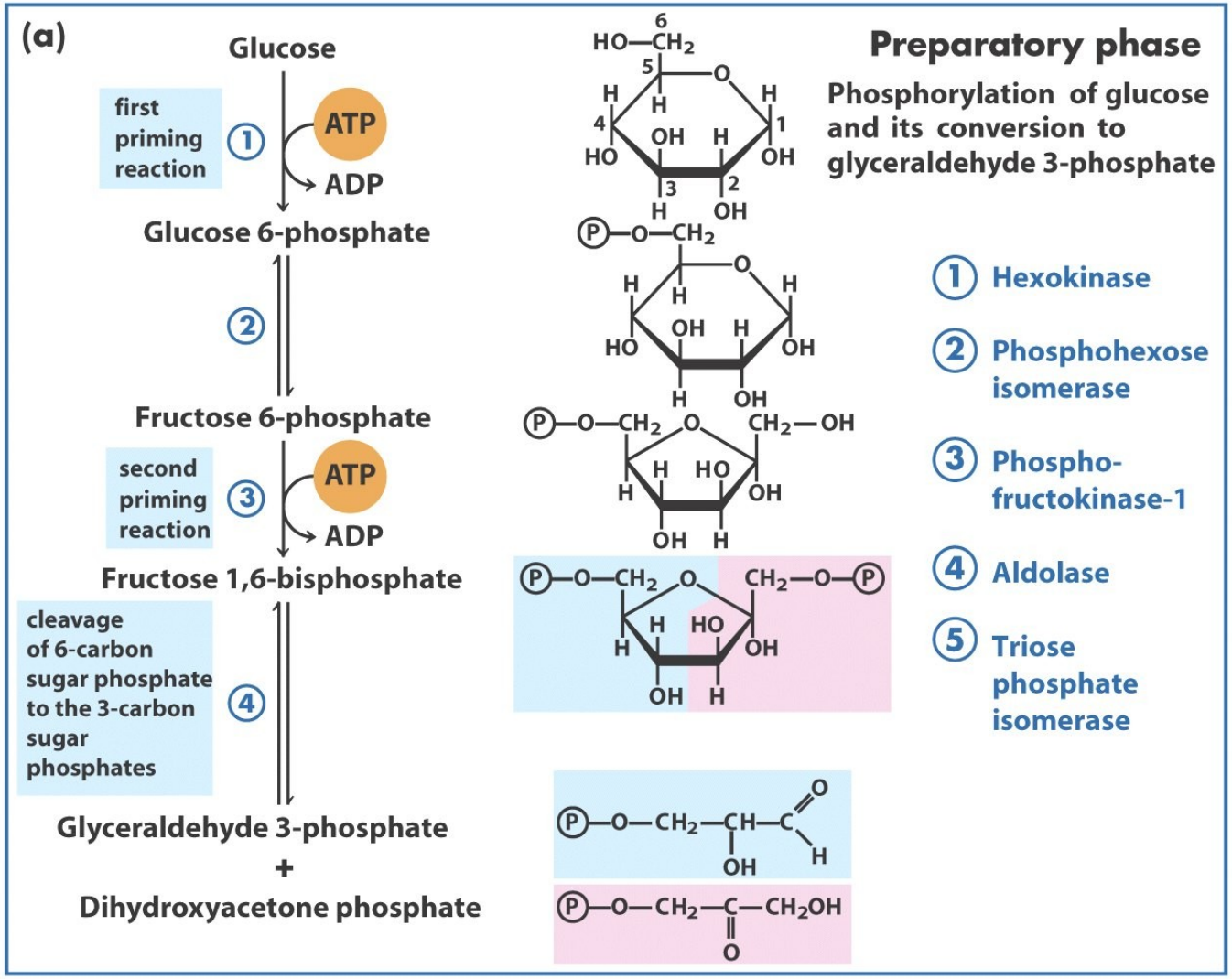


Ten steps of Glycolysis can be subdivided in two Phases:

I. The Preparatory Phase (steps 1-5)
- spend ATP
- glucose → 2 glyceraldehyde-3-phosphate

II. The Payoff Phase (steps 6-10)
- generate ATP & NADH
- 2 glyceraldehyde-3-phosphate → 2 pyruvate

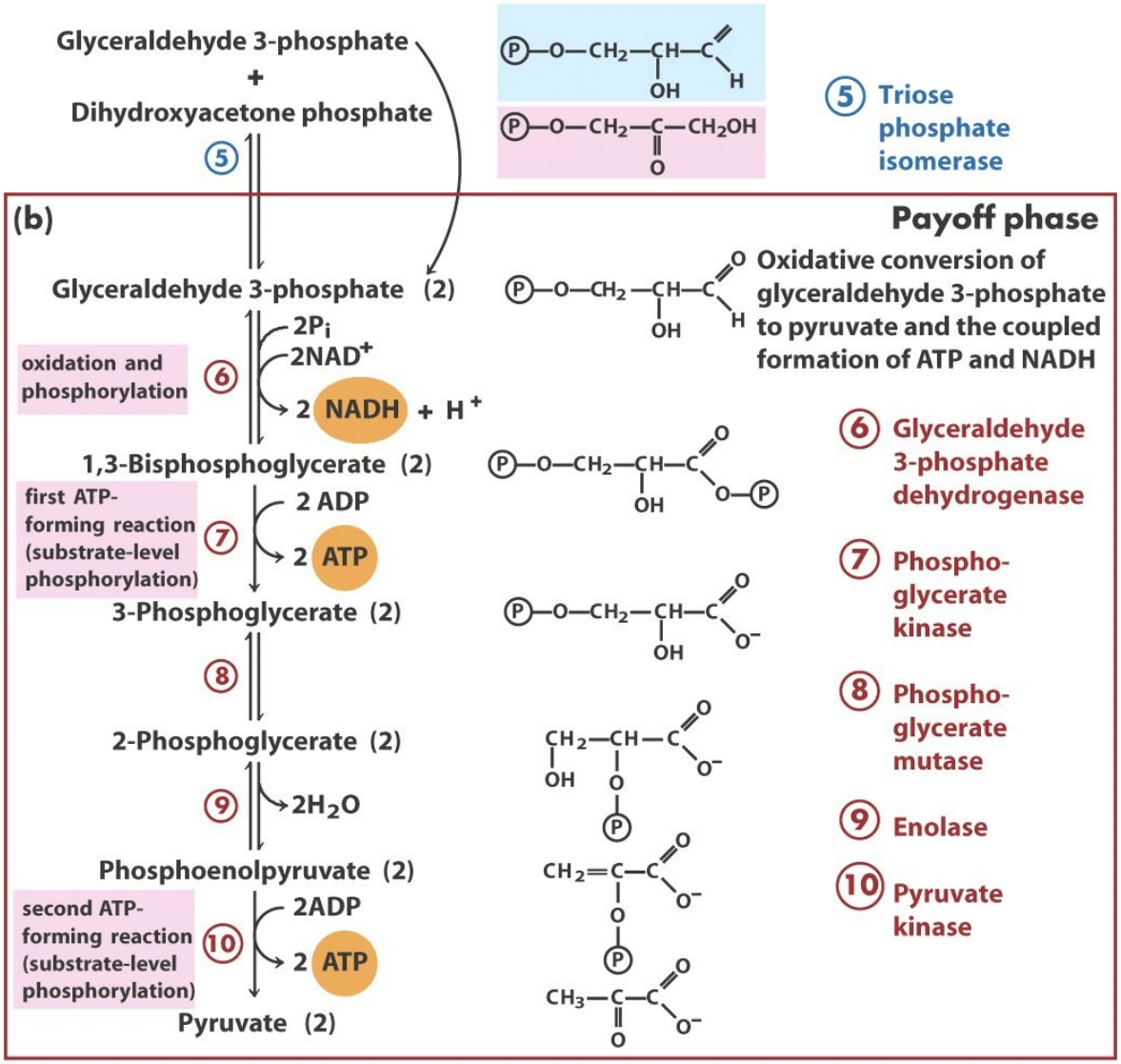
Preparatory Phase of Glycolysis



Enzymes
- 2 Kinases
- 2 Isomerases

- 1 Aldolase

Payoff Phases of Glycolysis



Enzymes
- 2 Kinases
- 1 Mutase

- 1 Dehydrogenase
- 1 Enolase

Yield (in energy equivalents) per glucose

University of Lethbridge

Chemical equation for glycolysis:

$$\text{Glucose} + 2NAD^+ + 2ADP + 2P_i \rightarrow 2 \text{ pyruvate} + 2NADH + 2H^+ + 2ATP + 2H_2O$$

Formation of 2x pyruvate, NADH and H^+ (energy released):

$$\text{Glucose} + 2NAD^+ \rightarrow 2 \text{ pyruvate} + 2NADH + 2H^+ \qquad \Delta G'^{\circ}_1 = -146 \text{ kJ/mol}$$

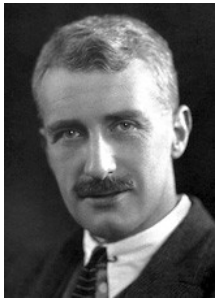
Formation of 2 ATP (energy cost):

$$2ADP + 2P_i \rightarrow 2ATP + 2H_2O \qquad \Delta G'^{\circ}_2 = 61.0 \text{ kJ/mol}$$

$$\Delta G'^{\circ}_S = \Delta G'^{\circ}_1 + \Delta G'^{\circ}_2 = -85 \text{ kJ/mol}$$

A Historical Perspective

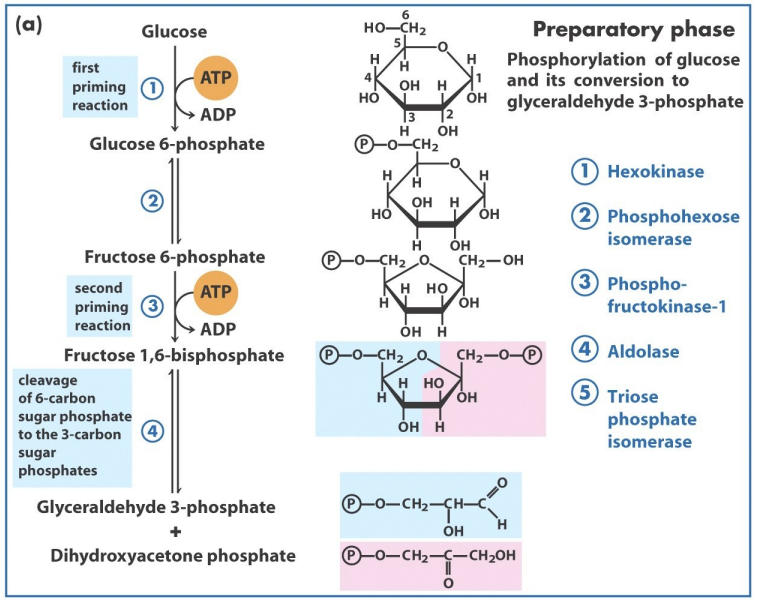
- 1854-1864: L. Pasteur establishes fermentation is caused by microorganisms.
- 1897: E. Buchner demonstrates cell-free yeast extracts carry out this process.
- 1905-1910: Arthur Harden and William Young discovered:
  - Inorganic phosphate is required for fermentation and is incorporated into fructose-1-6-bisphosphate
  - A cell-free yeast extract has a nondialyzable heat-labile fraction (zymase) and a dialyzable heat-stable fraction (cozymase).

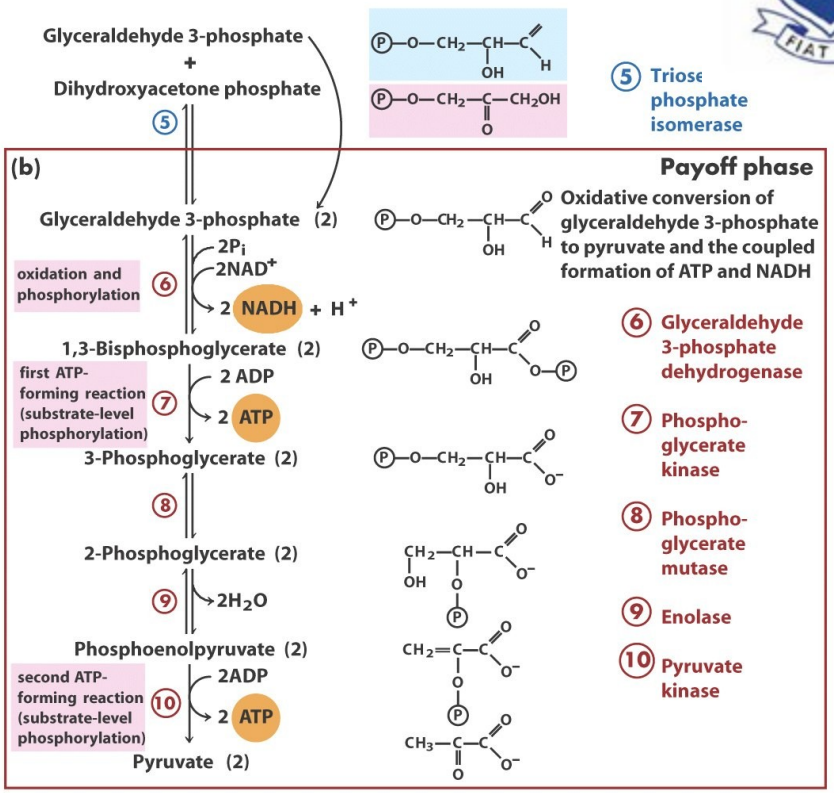


- By 1940: Elucidation of complete glycolytic pathway (Gustav Embden, Otto Meyerhof, and Jacob Parnas).

Otto Fritz Meyerhof & Archibald Vivian Hill *The Nobel Prize in Physiology or Medicine 1922*

Glycolysis





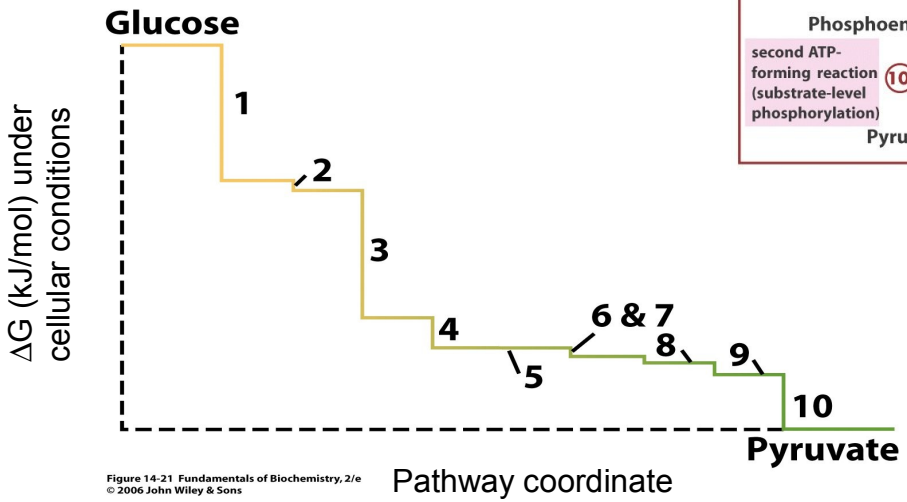
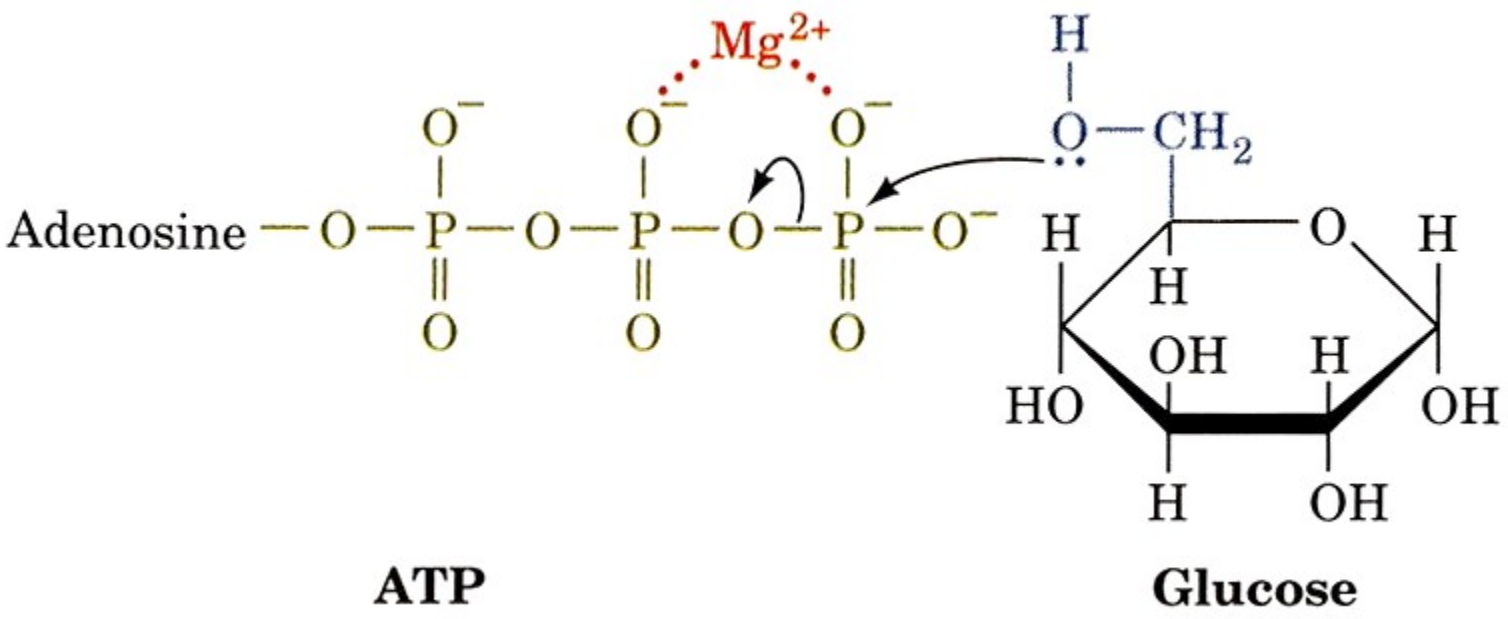


Figure 14-21 Fundamentals of Biochemistry, 2/e
© 2006 John Wiley & Sons

Hexokinase: First ATP Utilization

Reaction 1 : **Transfer of a phosphoryl group from ATP to glucose to form glucose 6-phosphate (G6P)**



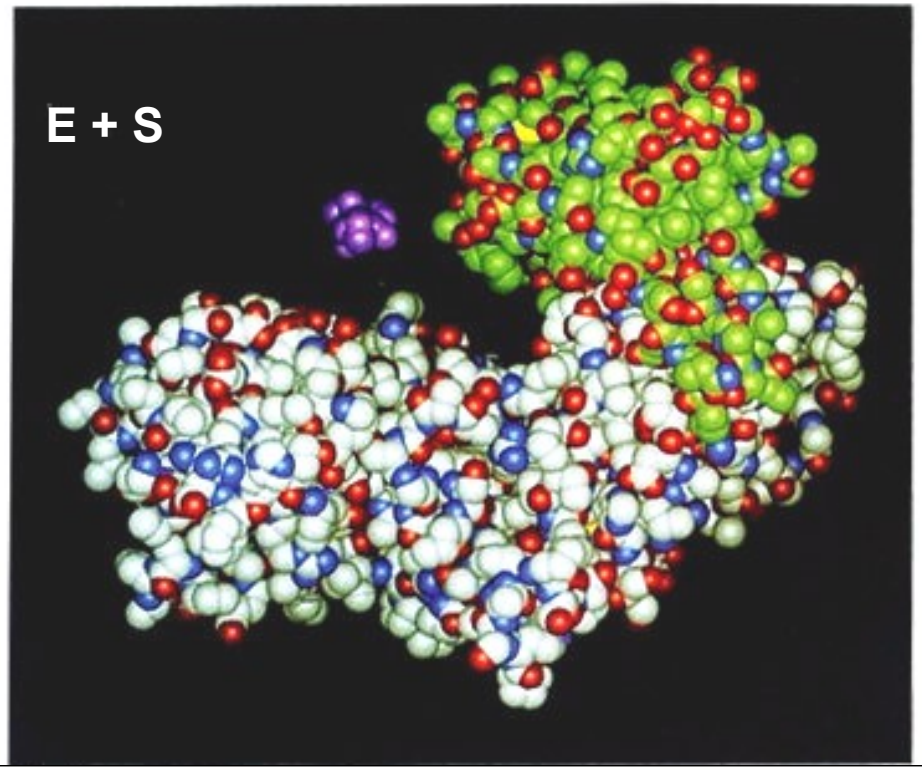
Irreversible under cellular conditions due to large, negative ΔG'

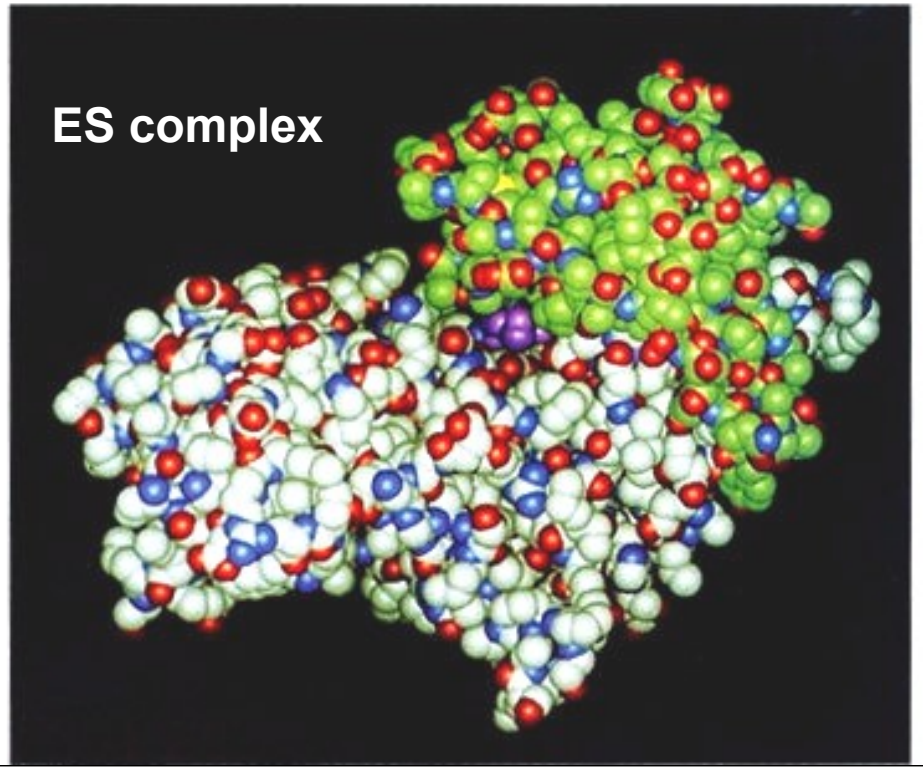
ΔG'° = -16.7 kJ/mol

Hexokinase: First ATP Utilization

The two domains (grey/green) swing together when bound to Pi

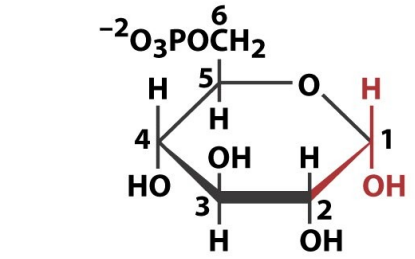
- excludes H_2O from active site (eg. electrostatic catalysis)





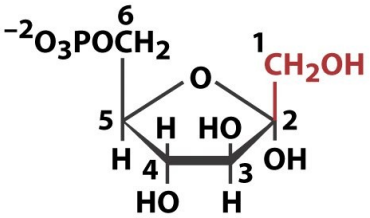
Phosphohexose Isomerase

Reaction 2: **Phosphohexose isomerase catalyzes the conversion of G6P to F6P (ie. aldose to ketose isomerisation)**



Glucose-6-phosphate (G6P)

phosphoglucose isomerase (PGI)

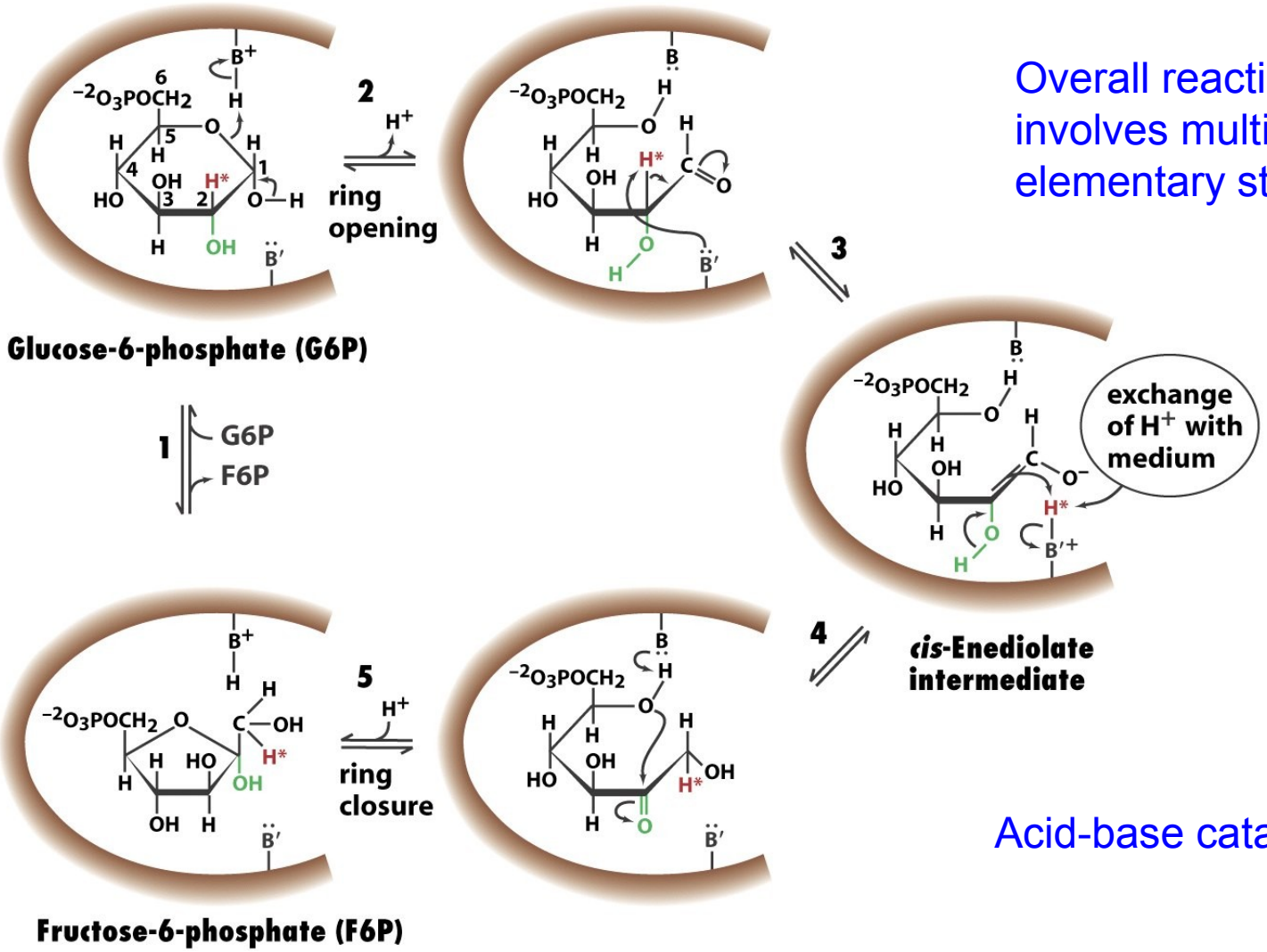


Fructose-6-phosphate (F6P)

Isomerases and mutases typically catalyze reactions with small $\Delta G'^{o}$ (and $\Delta G'$)

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

Phosphohexose Isomerase



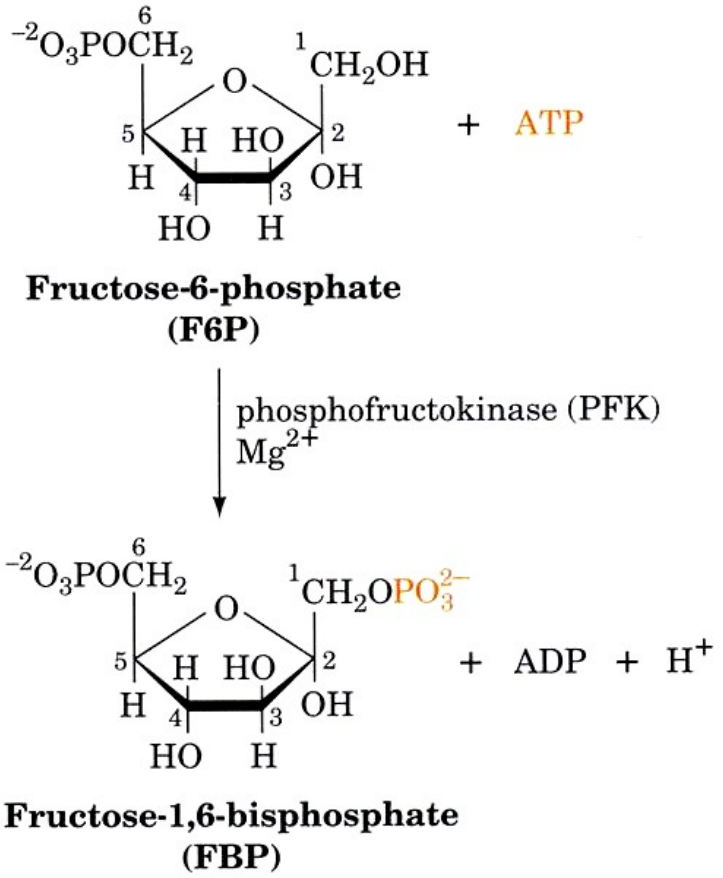
Overall reaction involves multiple (4) elementary steps

Acid-base catalysis

PFK-1: Second ATP Utilization

Reaction 3: **Phosophofructokinase-1 (PFK-1) phosphorylates fructose-6-phosphate (F6P)**

PFK-1 plays a central role in control of glycolysis as it catalyzes one of the pathway's rate-determining reactions.



Irreversible under cellular conditions due to large, negative ΔG'

ΔG'° = -14.2 kJ/mol

Aldolase

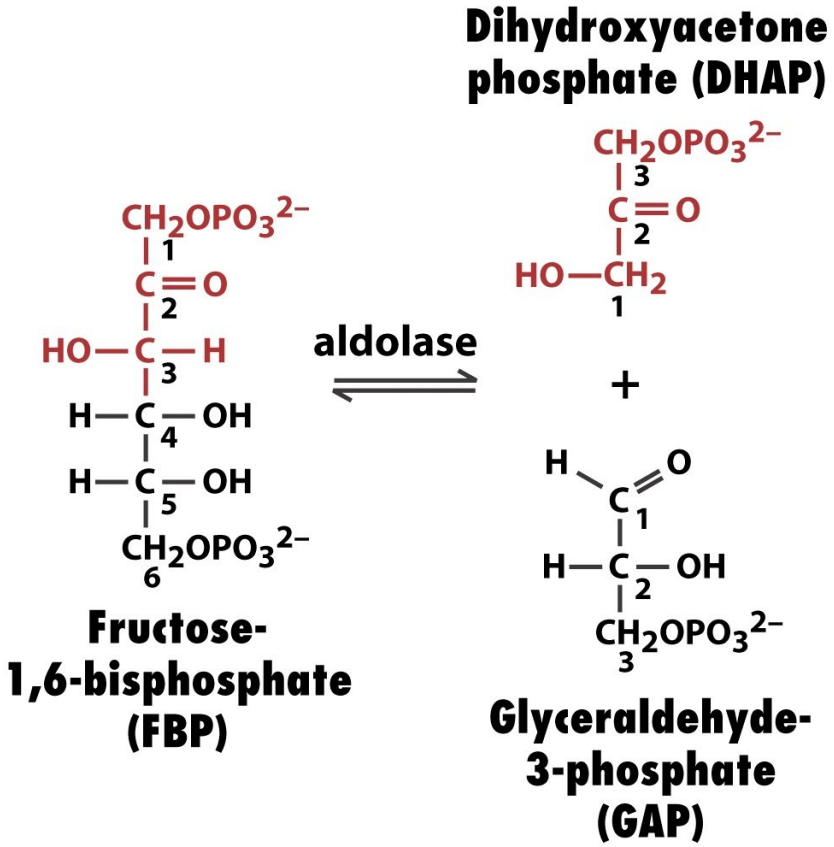
Reaction 4: **Aldolase catalyzes cleavage of fructose-1,6-bisphosphate (FBP)**

How is the large, unfavourable ΔG'° for this reaction overcome?

The [Product]/[Substrate] is kept very small

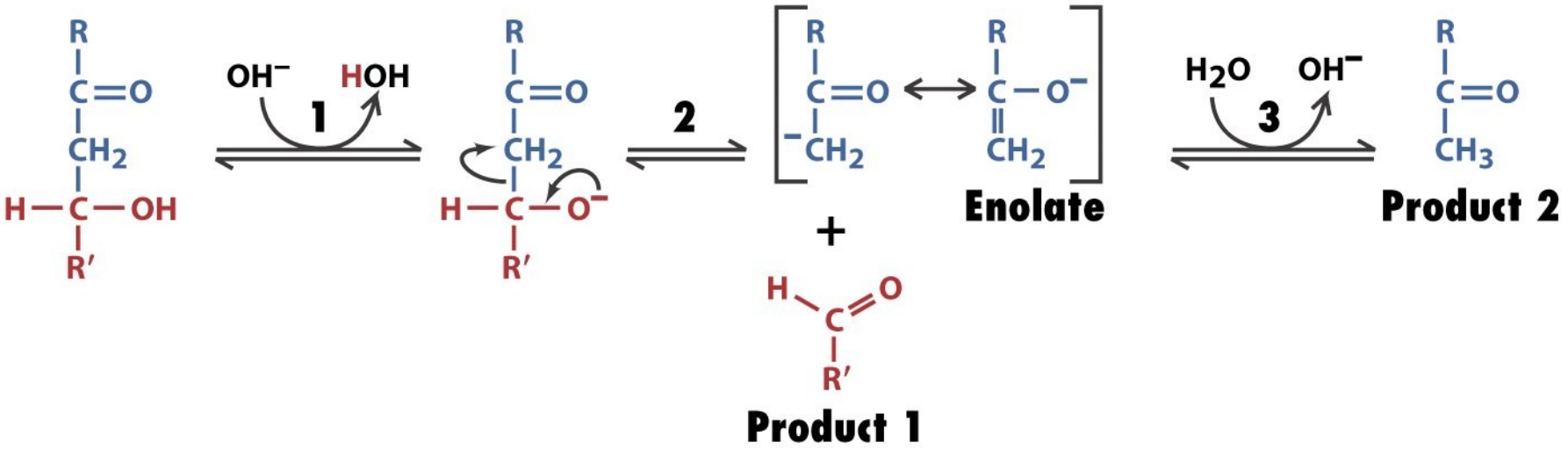
Enzymes operating far from their equilibrium state are regulatory targets

ΔG'° = 23.8 kJ/mol



Retro Aldol Reaction

The aldolase mechanism is similar to the retro-aldol mechansim in organic chemistry

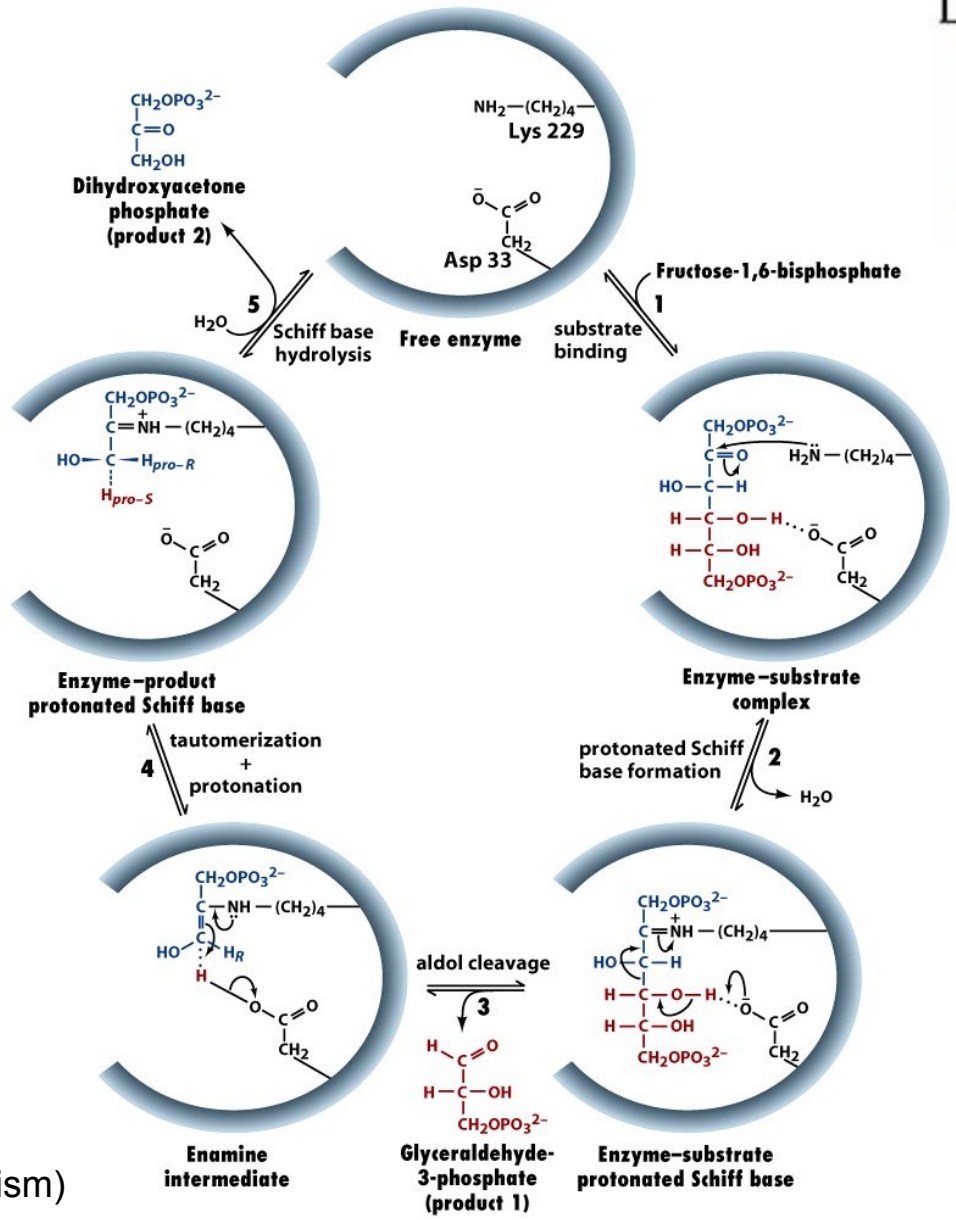


How could (and does) aldolase enhance the rate of this reaction?

Stabilize the carbanion !

(Class I) Aldolase Reaction Mechanism

Formation of a protonated Schiff's base (Lys 229) that stabilizes the carbanion/enamine

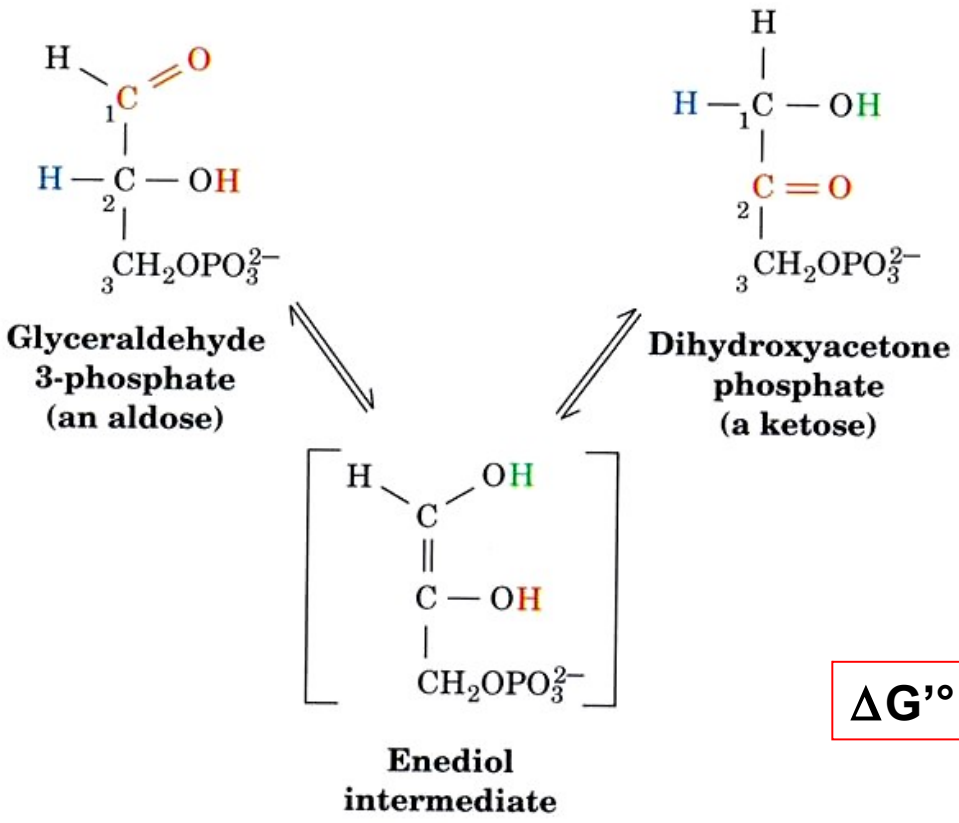


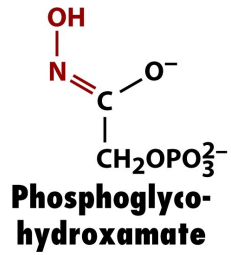
Class I Aldolase (Schiff's base mechanism)
Class II Aldolase (Metalloenzyme)

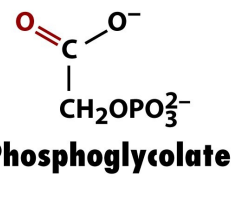
Triose Phosphate Isomerase

Reaction 5: Interconversion of the triose phosphates

Only G3P continues along the glycolytic pathway. DHAP (dihydroxyacetonephosphate) is rapidly and reversible converted to G3P by TPI (aka TIM)







Inhibitors used to study TPI mechanism

$\Delta G'° = 7.5$ kJ/mol

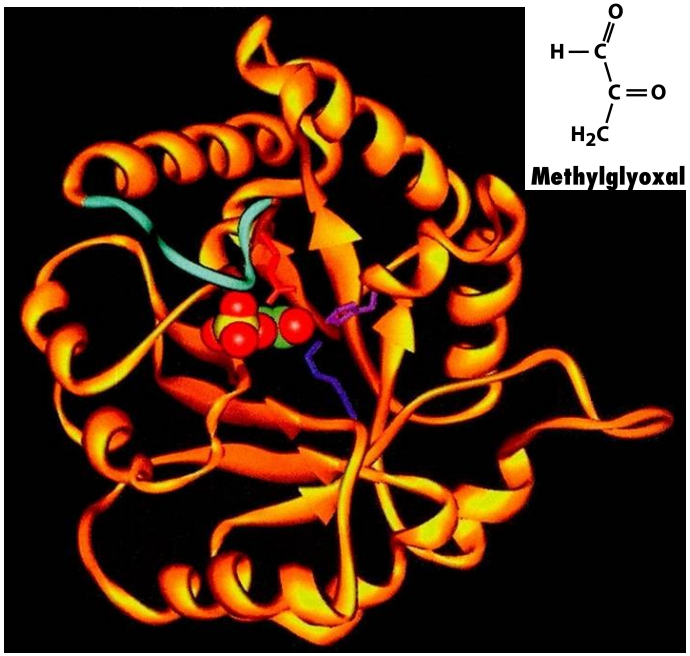
Triose Phosphate Isomerase

Ribbon diagram of **TPI** (**TIM**) in complex with its transition state analog 2-phosphoglycolate.

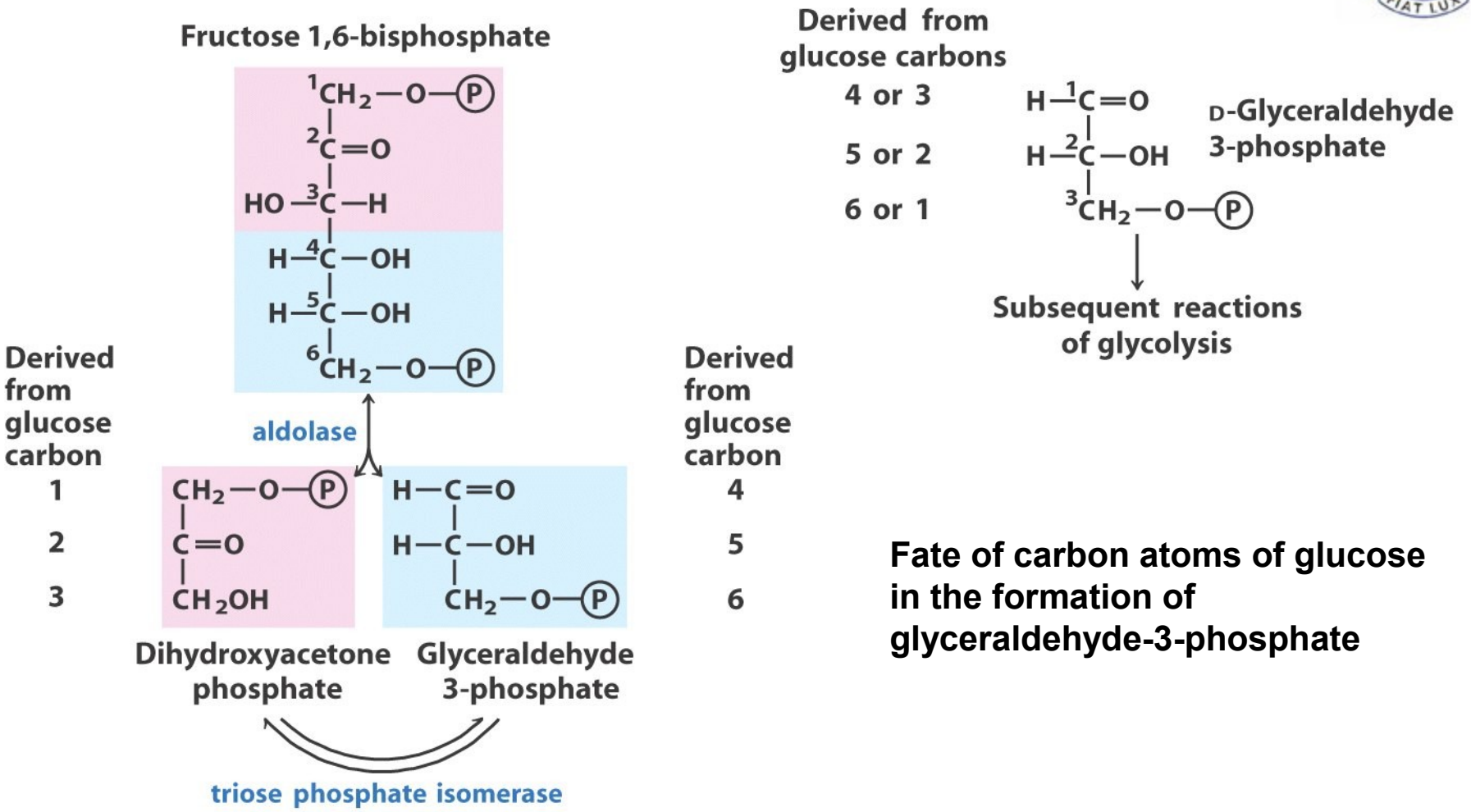
Flexible loop (light blue) makes a hydrogen bond with the phosphate group of the substrate.

Removal of loop (mutagenesis) does not impair substrate binding but reduces catalytic rate by 10^5 fold.

stereoelectronic control
(electrostatic catalysis)

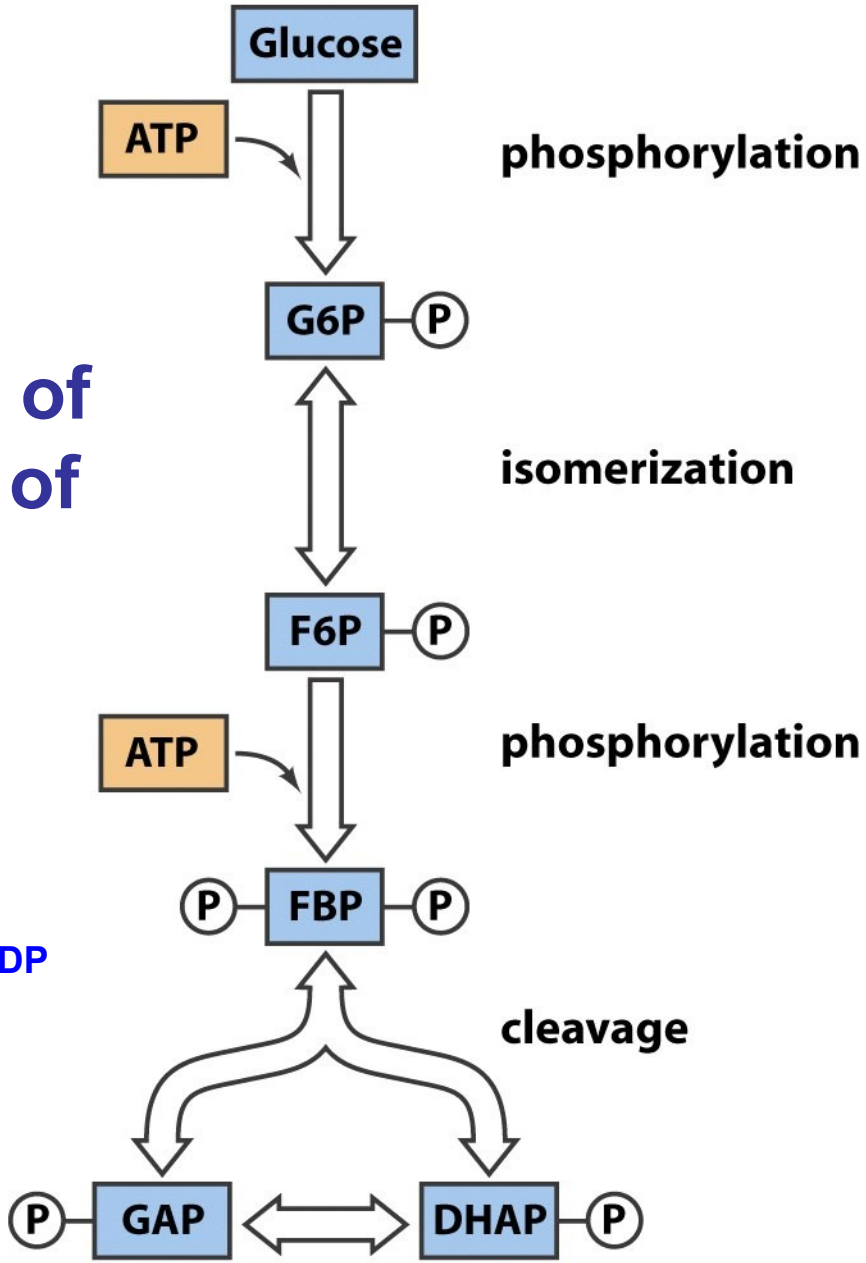


Summary of Reaction 4 & 5



Fate of carbon atoms of glucose in the formation of glyceraldehyde-3-phosphate

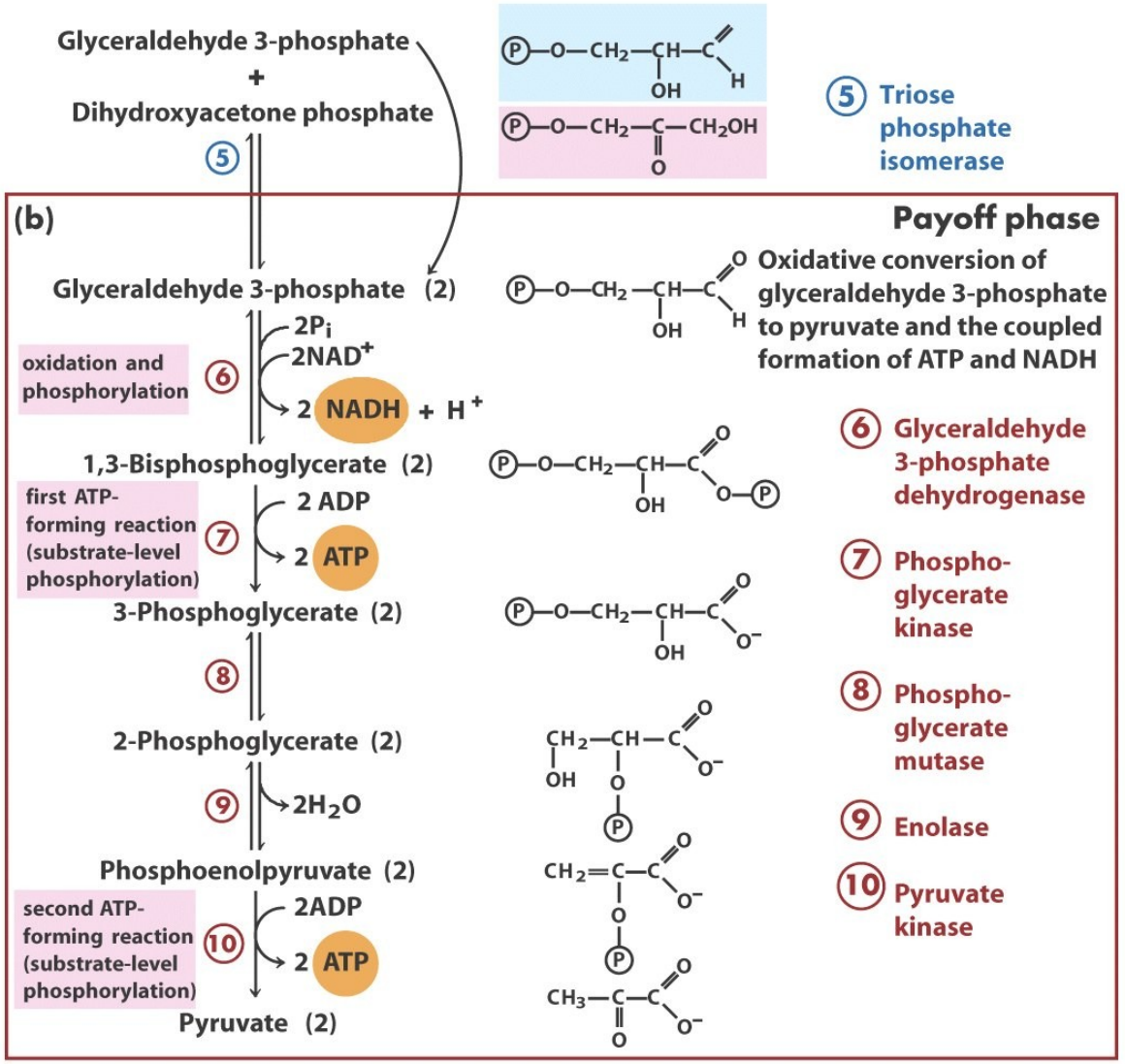
'Cartoon' summary of preparatory phase of glycolysis



Overall Reaction for Preparatory Phase of Glycolysis

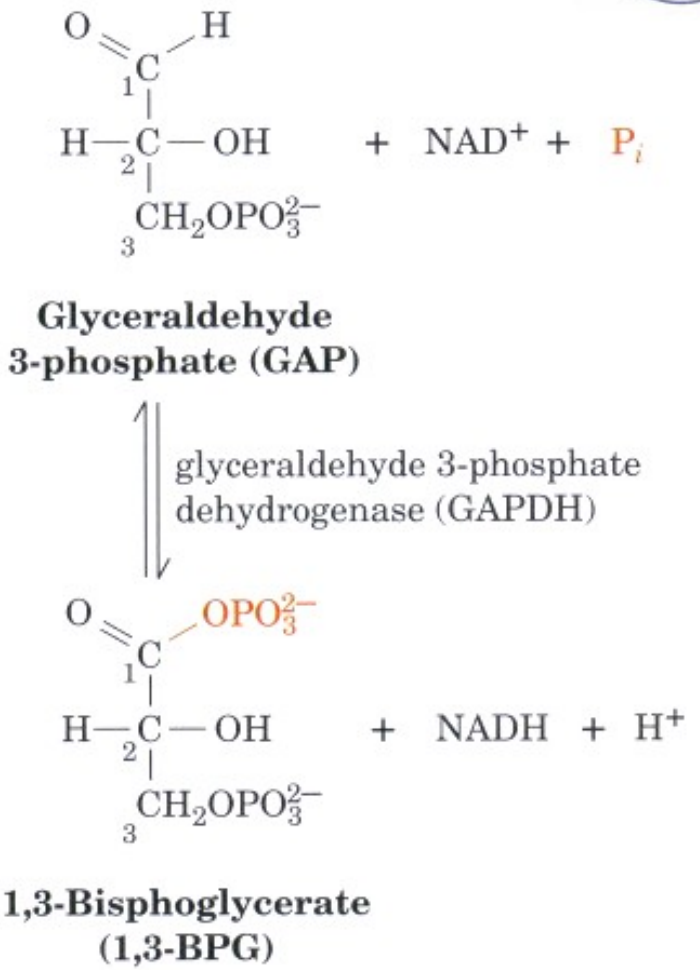
Glucose + 2 ATP ⇨
2 Glyceraldehyde-3-phosphate + 2 ADP

The Payoff Phase



Glyceraldehyde-3-Phosphate Dehydrogenase

Reaction 6:
Glyceraldehyde-3-phosphate dehydrogenase forms the first "high-energy" intermediate.



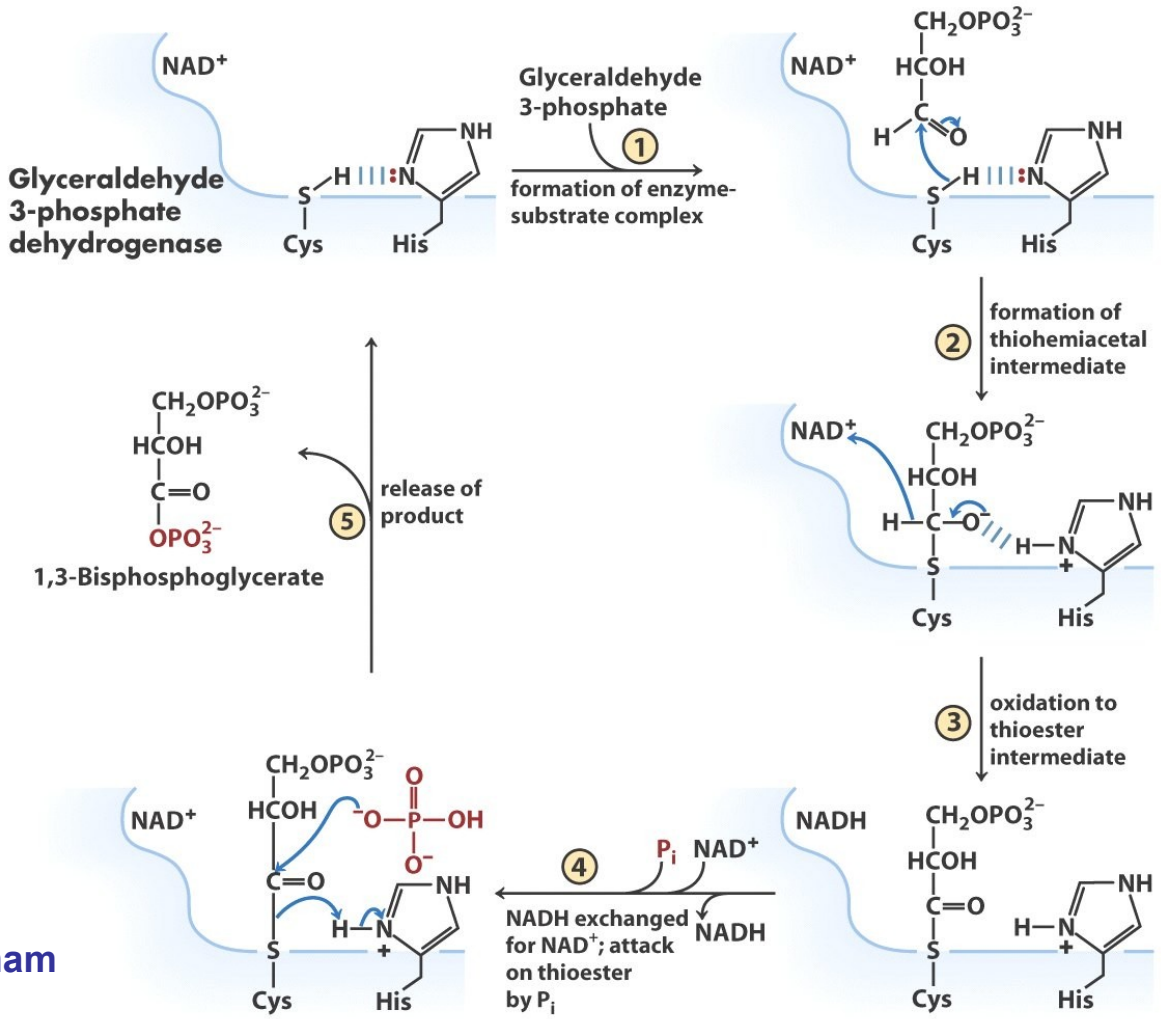
Substrate Level Phosphorylation

P_i is the substrate !!

High energy phosphodiester and NADH produced without expending ATP !!!

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

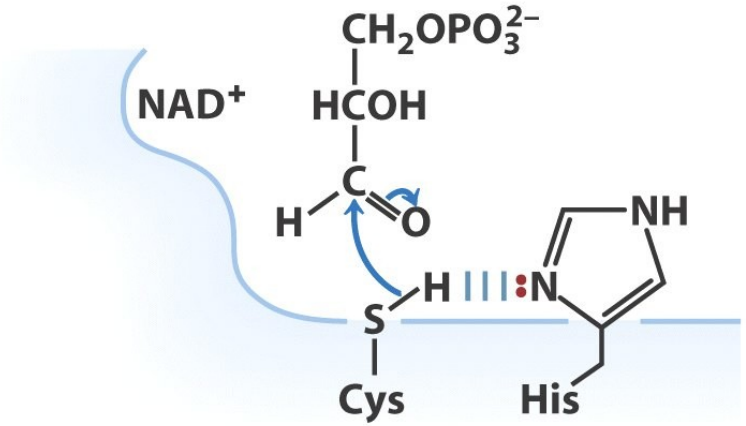
Glyceraldehyde 3-Phosphate Dehydrogenase Reaction Mechanism



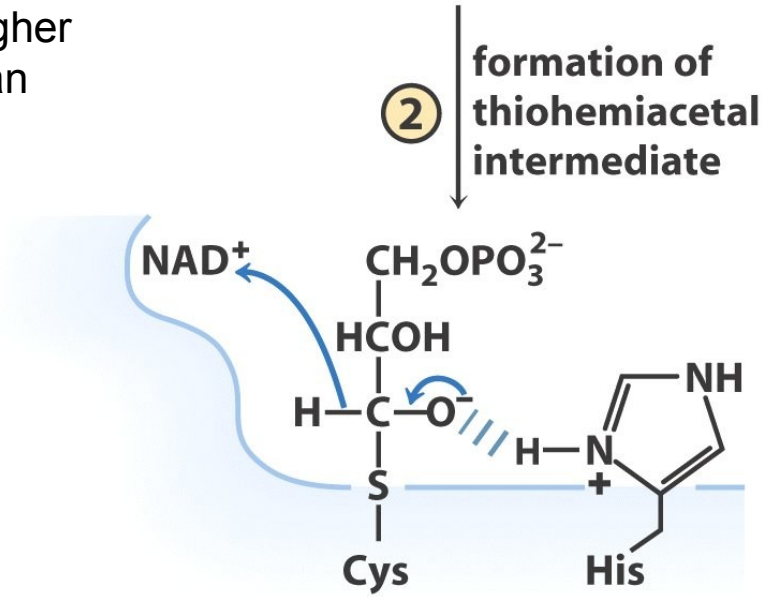
David Trentham
Mechanism

Glyceraldehyde 3-Phosphate Dehydrogenase (covalent intermediate)

Covalent intermediate:

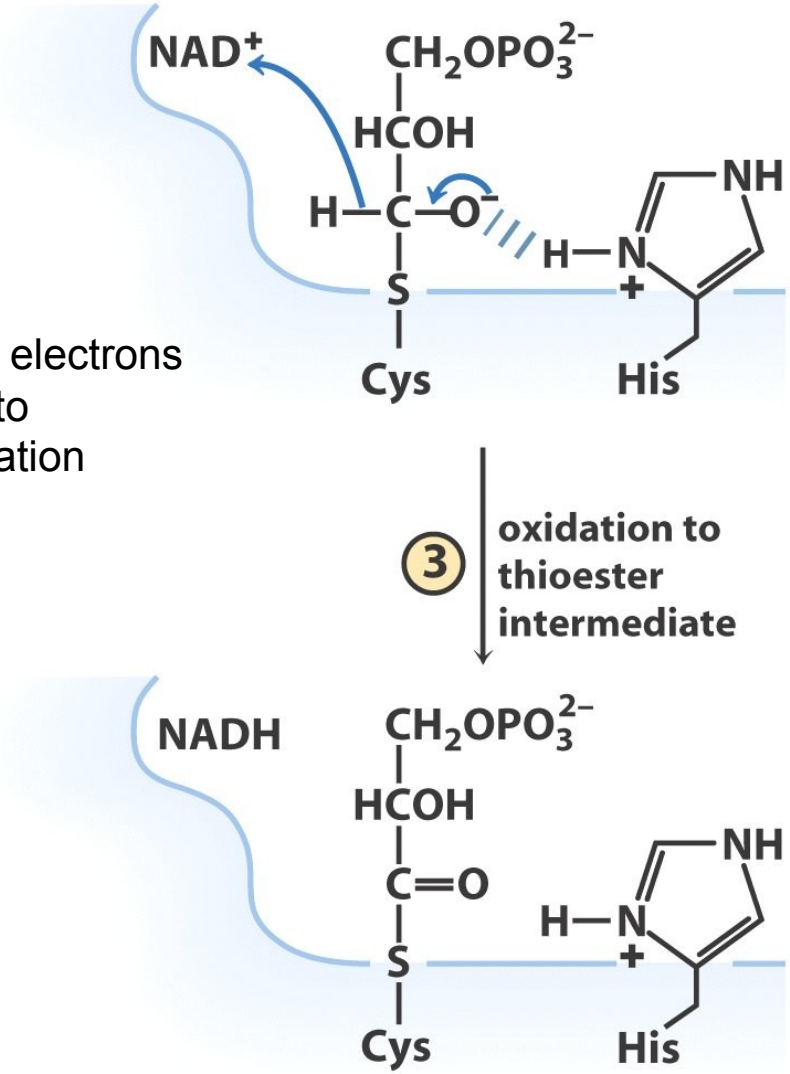


thiohemiacetals are higher energy compounds than hemiacetals

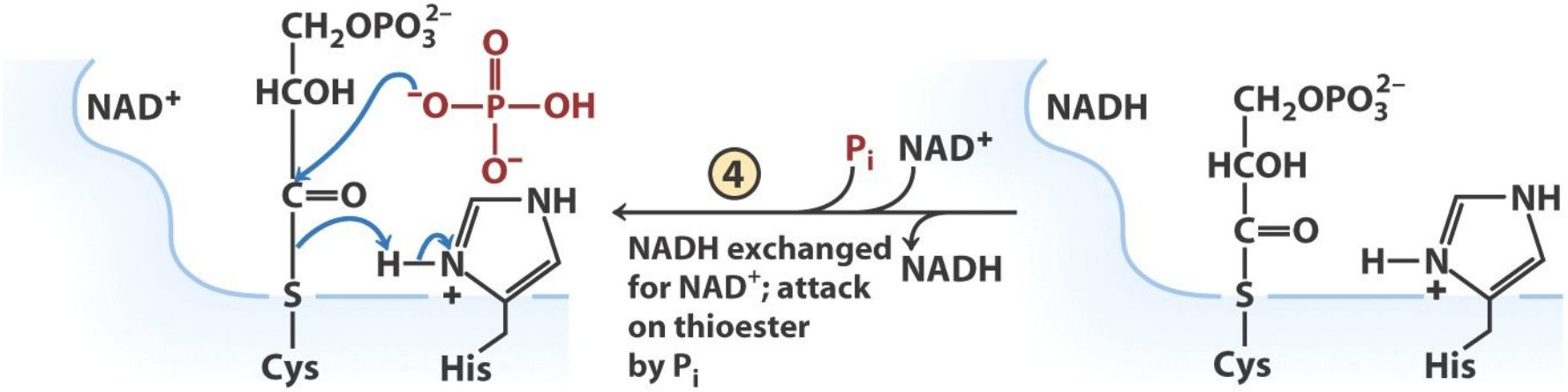


Glyceraldehyde 3-Phosphate Dehydrogenase (oxidation step)

Bound NAD^+ accepts electrons
That are funnelled into
oxidative phosphorylation

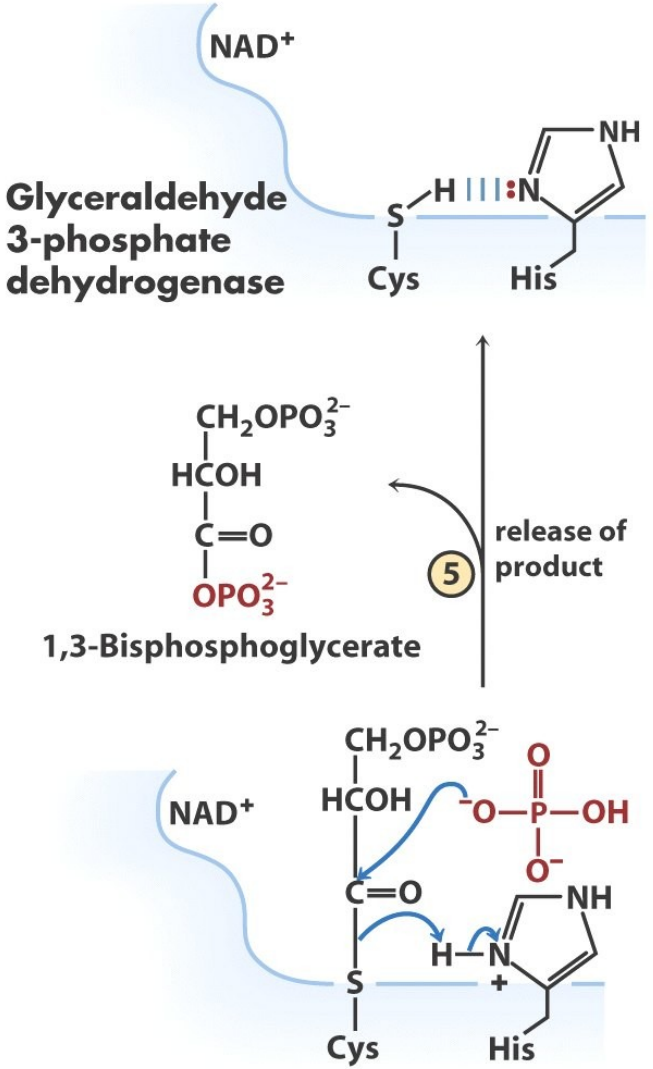


Glyceraldehyde 3-Phosphate Dehydrogenase (substrate level phosphorylation)



Thioester is cleaved by Pi producing a high energy compound:
1,3-bisphosphoglycerate

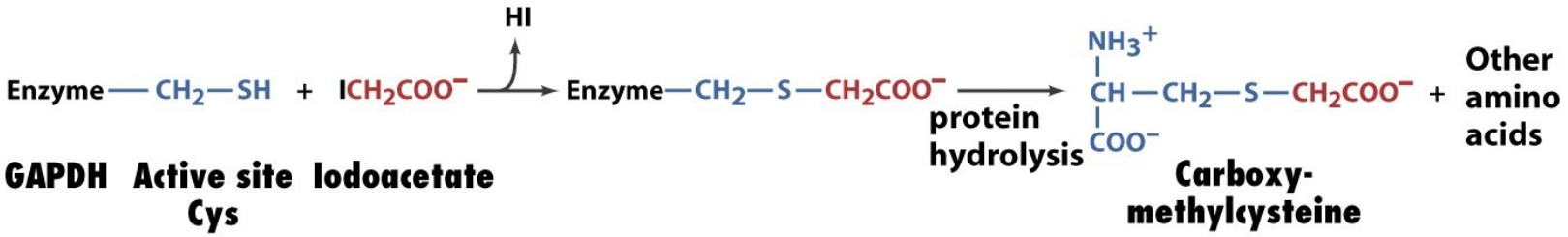
Glyceraldehyde 3-Phosphate Dehydrogenase (product release)



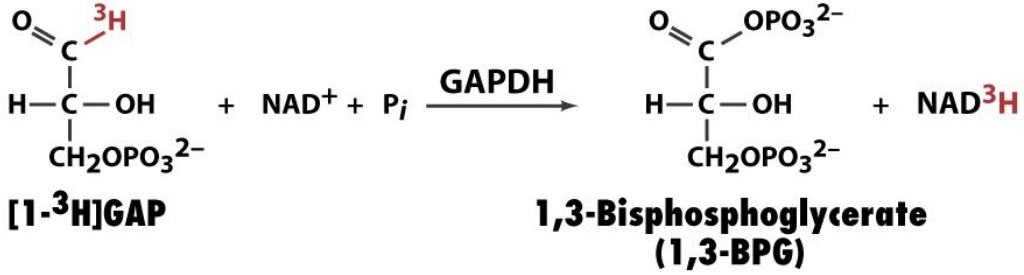
Glyceraldehyde-3-Phosphate Dehydrogenase

Mechanistic Studies:

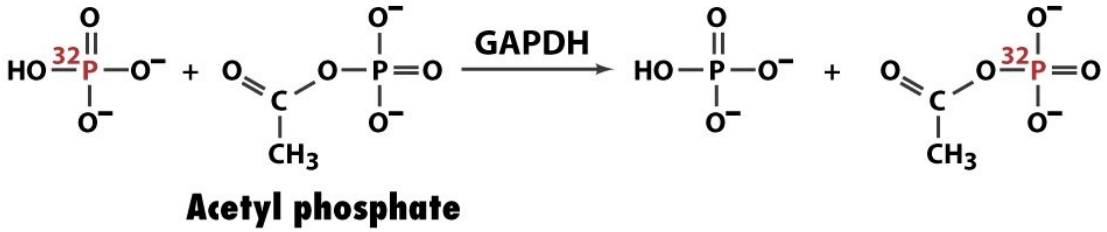
(a) Iodoacetate inactivates enzyme by modification of active site cysteine



(b) Aldehyde group provides H^+ for NAD^+ reduction

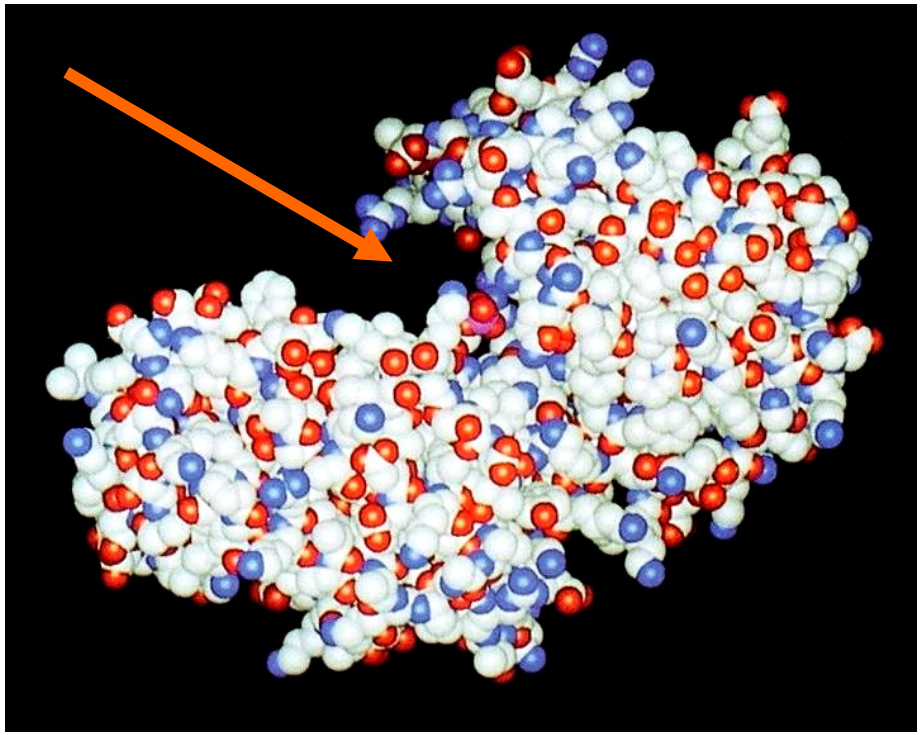


(c) P_i is substrate



Phosphoglycerate Kinase: First ATP Generation

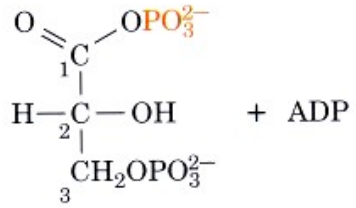
Upon substrate binding, the two domains of PGK swing together, providing a water-free environment (just like hexokinase)



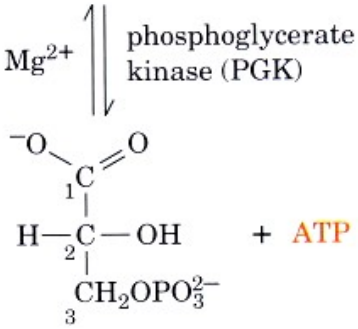
Glycolysis:

Two kinase enzymes (hexokinase and PGK) are homologs with different substrate specificities

Phosphoglycerate Kinase: First ATP Generation



1,3-Bisphosphoglycerate (1,3-BPG)

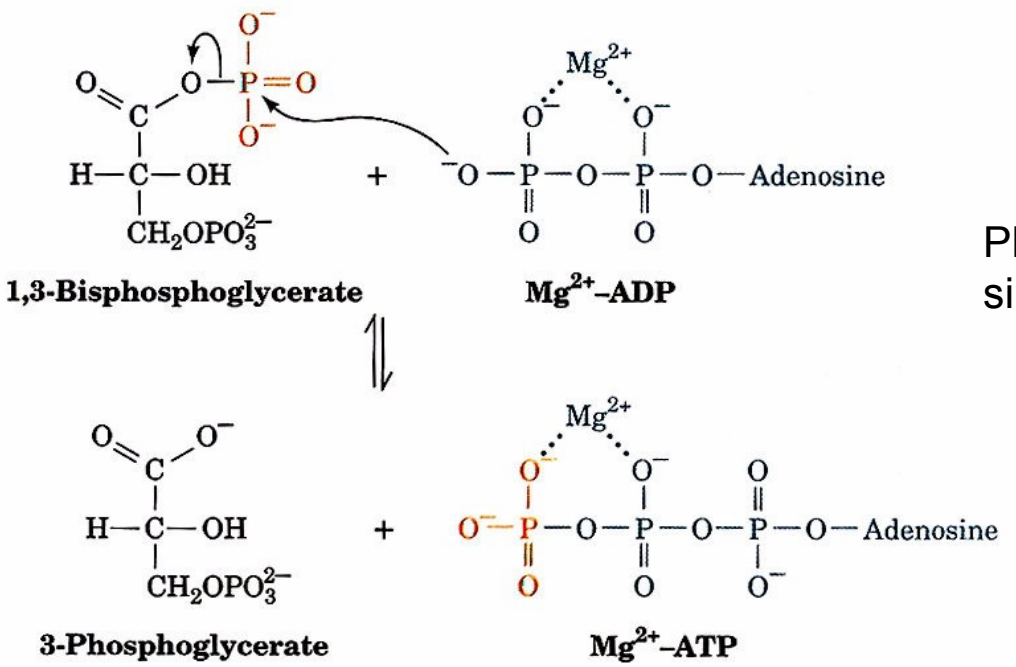


3-Phosphoglycerate (3PG)

1,3-bisphosphoglycerate is a higher energy compound than ATP so the reaction has a large favourable free energy change

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

Mechanism of PGK reaction



Phosphotransferase reaction similar to hexokinase (and PFK)

Energetics of the Glyceraldehyde-3-Phosphate Dehydrogenase:Phosphoglycerate Kinase Reaction Pair

$$\text{GAP} + P_i + \text{NAD}^+ \longrightarrow \text{1,3-BPG} + \text{NADH} \qquad \Delta G^{\circ\prime} = +6.7\ \text{kJ} \cdot \text{mol}^{-1}$$

$$\text{1,3-BPG} + \text{ADP} \longrightarrow \text{3PG} + \text{ATP} \qquad \Delta G^{\circ\prime} = -18.8\ \text{kJ} \cdot \text{mol}^{-1}$$

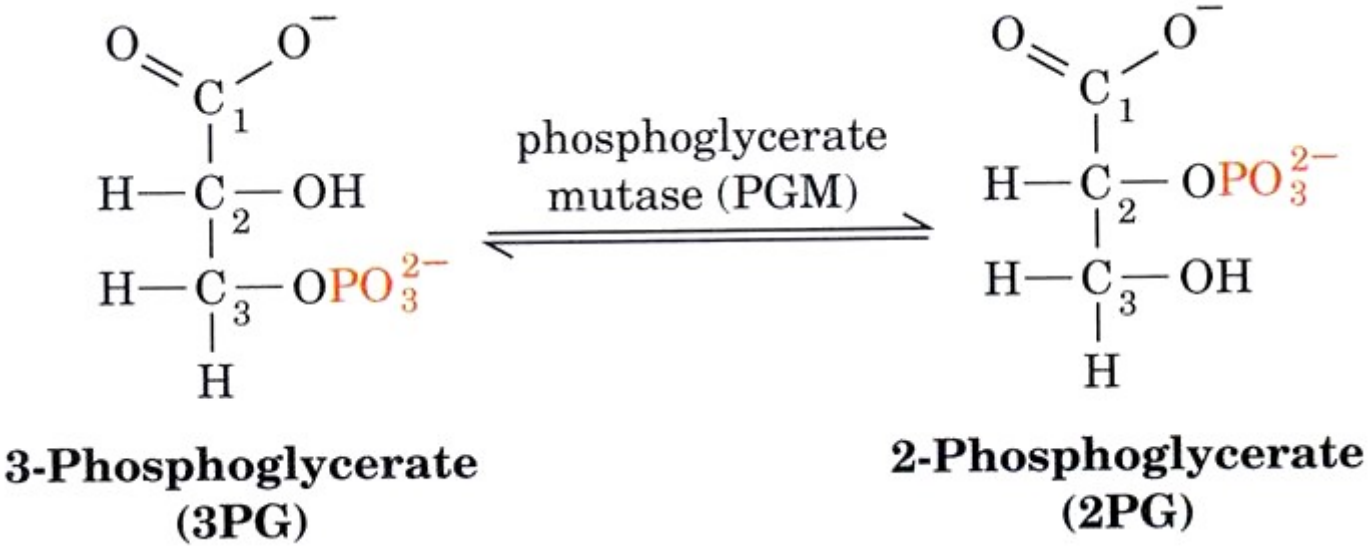
$$\text{GAP} + P_i + \text{NAD}^+ + \text{ADP} \longrightarrow \text{3PG} + \text{NADH} + \text{ATP} \qquad \Delta G^{\circ\prime} = -12.1\ \text{kJ} \cdot \text{mol}^{-1}$$

Coupling the two steps of the pathway:

Under standard condition: 1,3-BPG phosphotransfer drives the coupled reaction forming NADH and ATP.

Phosphoglycerate Mutase

Reaction 8: **Catalyzes a reversible shift of the phosphoryl group between C-2 and C-3 of glycerate;**
Mg^{2+} and 2,3-bisphosphoglycerate are essential.



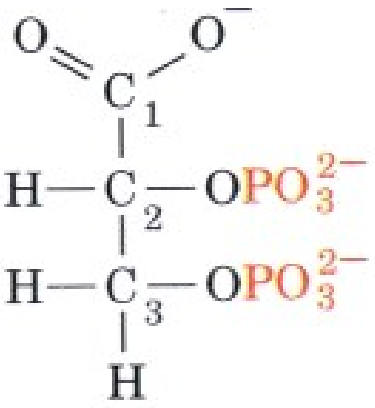
3-Phosphoglycerate (3PG)

2-Phosphoglycerate (2PG)

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

Reaction Mechanism of PGM

Catalytic amounts of 2,3-bisphosphoglycerate are required for enzymatic activity.

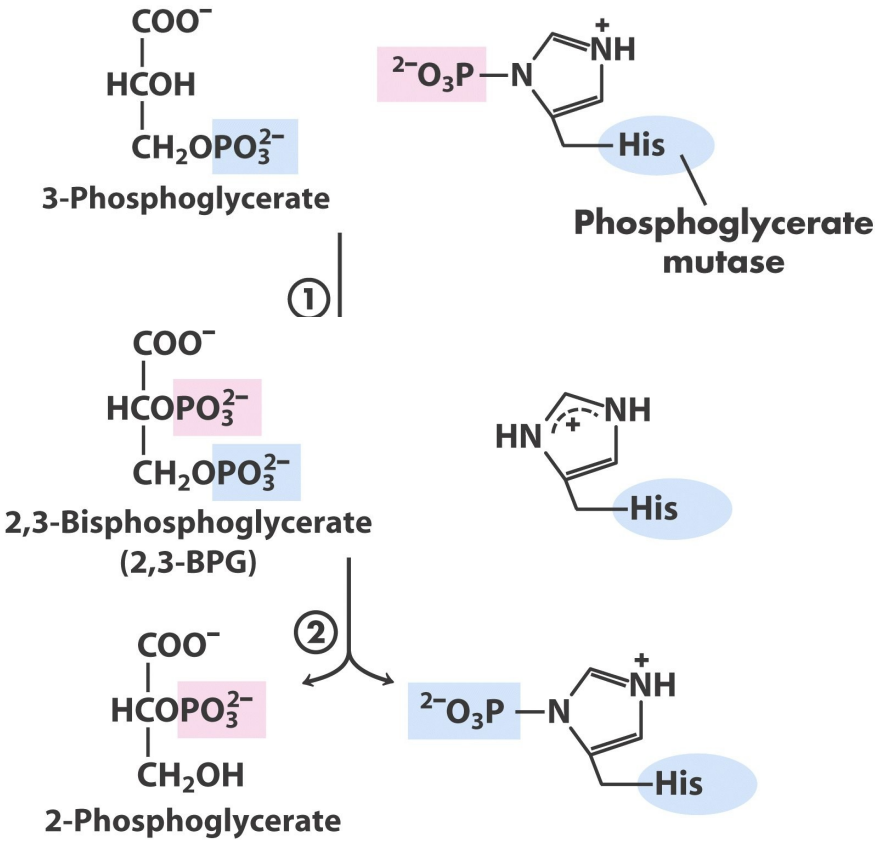


2,3-Bisphosphoglycerate (2,3-BPG)

2,3-bisphosphoglycerate 'activates' PGM by phosphorylating active site histidine

ie. Enzyme is inactive until phosphorylated

Reaction Mechanism of PGM



Step 1:

Phosphohistidine transfers phosphoryl to 3PG forming 2,3-BPG

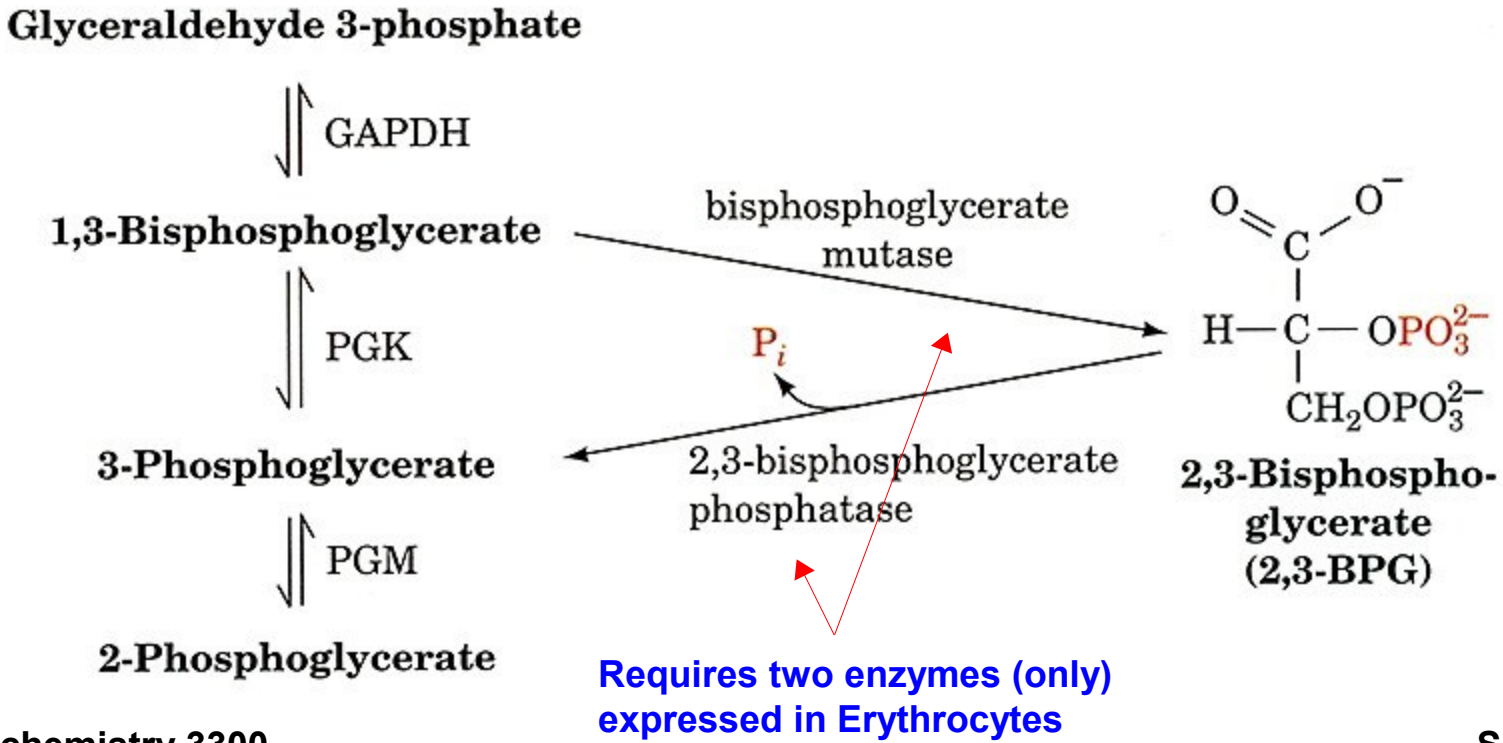
Step 2:

2,3-BPG transfers phosphoryl (3C) to His forming 2BPG and phosphorylated His

Gycolysis Influences Oxygen Transport

2,3-BPG binds to deoxyhemoglobin and alters (decreases) oxygen binding affinity.

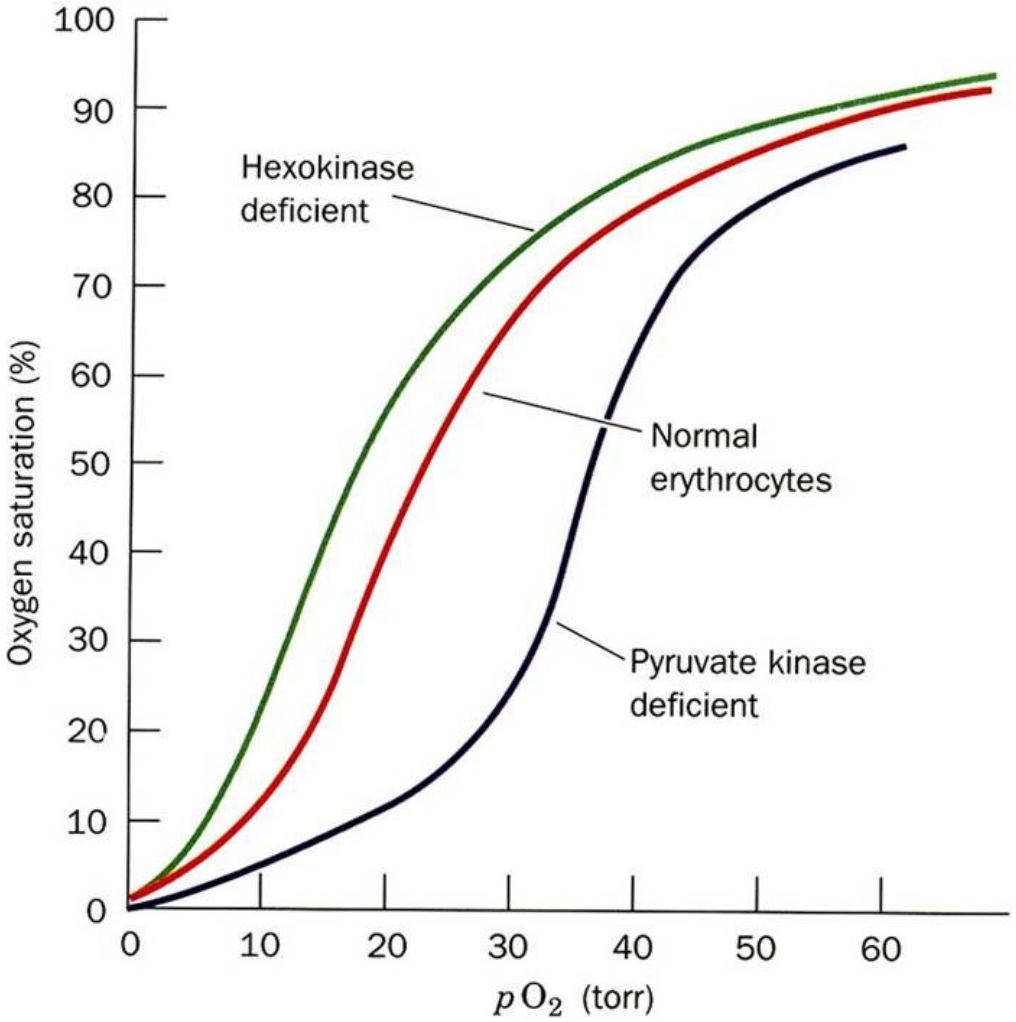
<u>Erythrocytes</u> synthesize and degrade 2,3-BPG using a 'detour' within the glycolytic pathway.



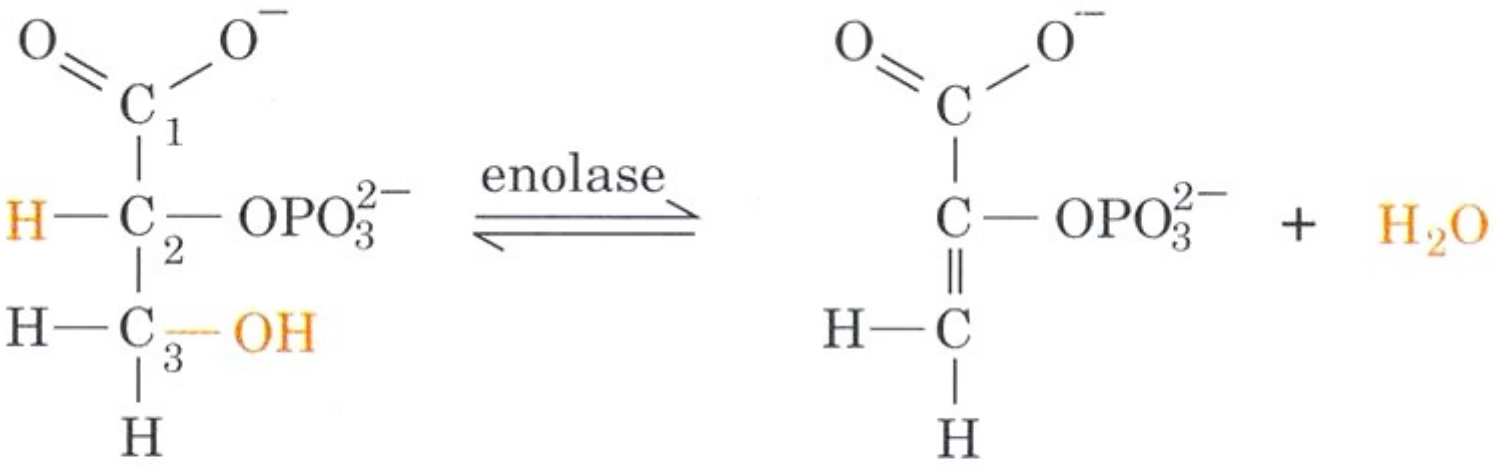
Gycolysis Influences Oxygen Transport

Lower [2,3-BPG] in erythrocytes resulting from hexokinase-deficiency results in increased hemoglobin oxygen affinity.

Higher [2,3-BPG] in erythrocytes resulting from PK-deficiency results in decreased hemoglobin oxygen affinity.



Enolase: Second "High Energy" Intermediate

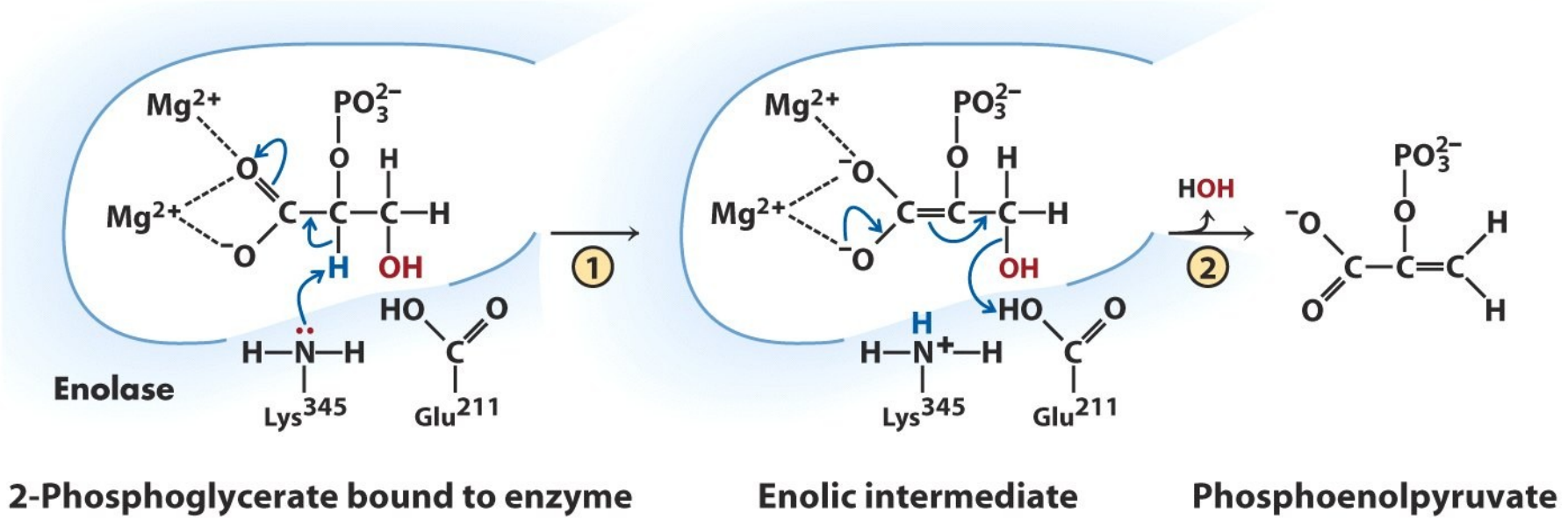


2-Phosphoglycerate (2PG)

Phosphoenolpyruvate (PEP)

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

Enolase Reaction Mechanism



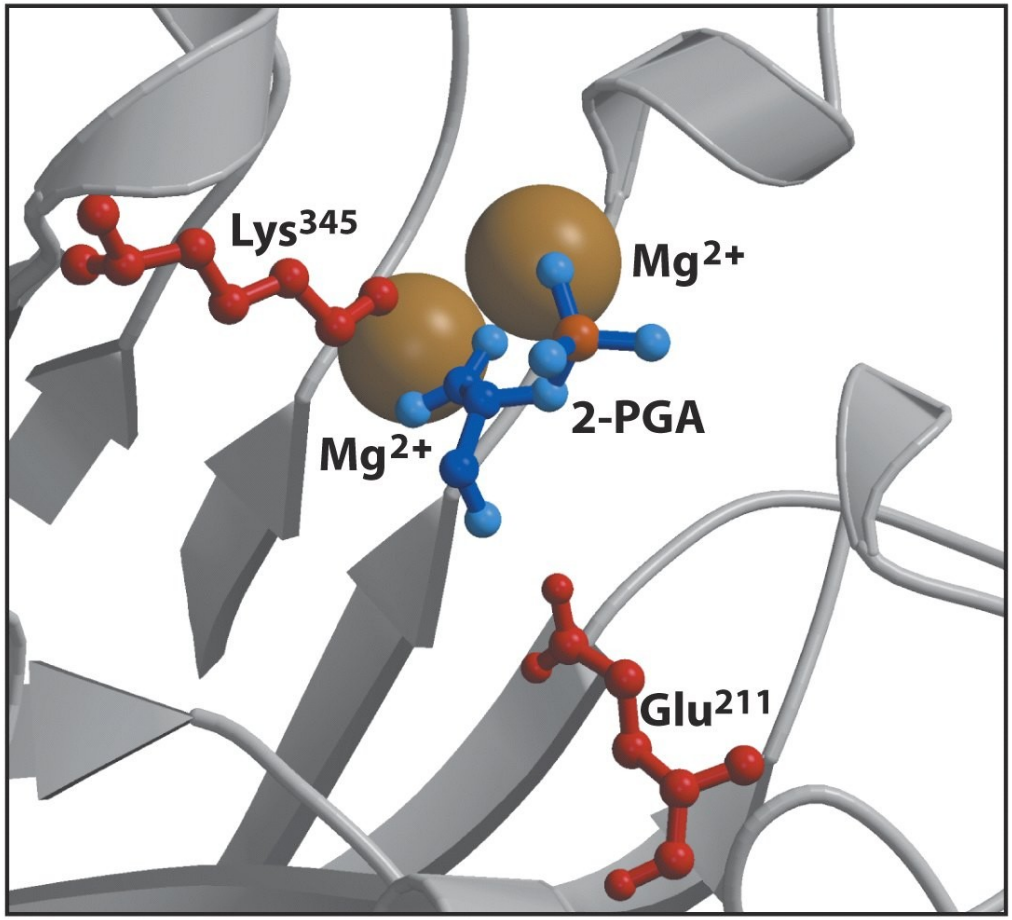
2-Phosphoglycerate bound to enzyme | Enolic intermediate | Phosphoenolpyruvate

Can be inhibited by F^-. Why?
F^- binds to strongly metals

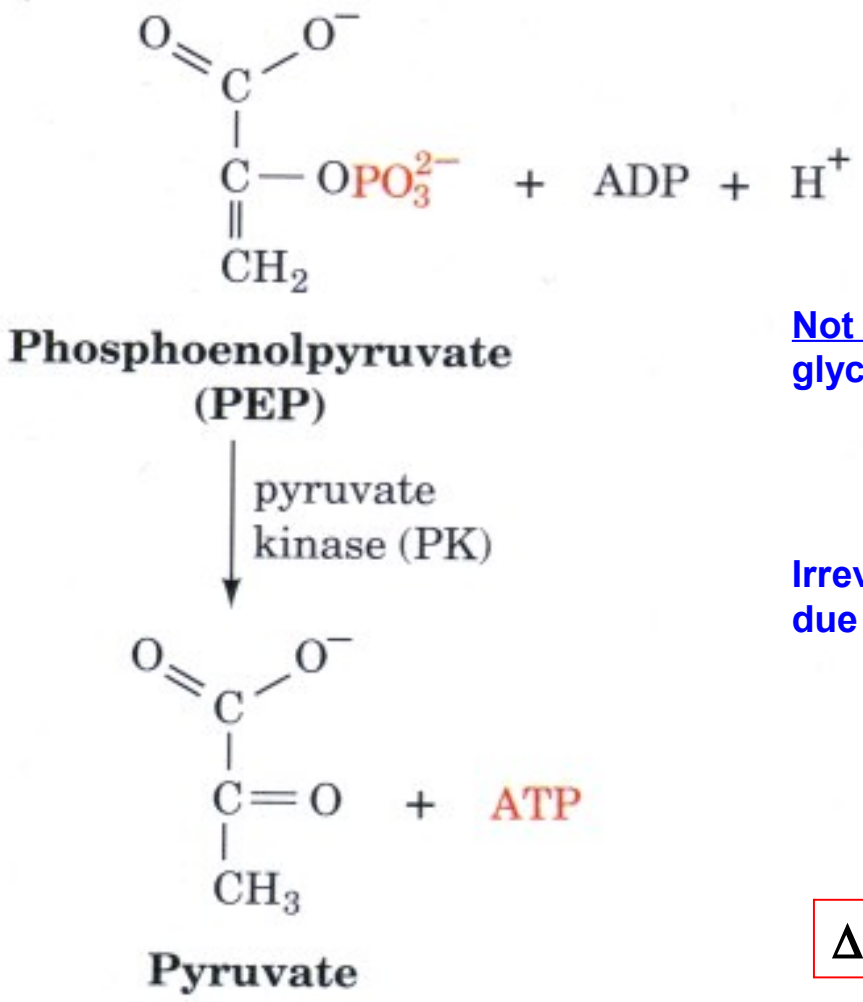
Overall reaction
is a dehydration

Enolase Reaction Mechanism

Structure of enolase catalytic center (PDB ID 1ONE)



Pyruvate Kinase : Second ATP Generation

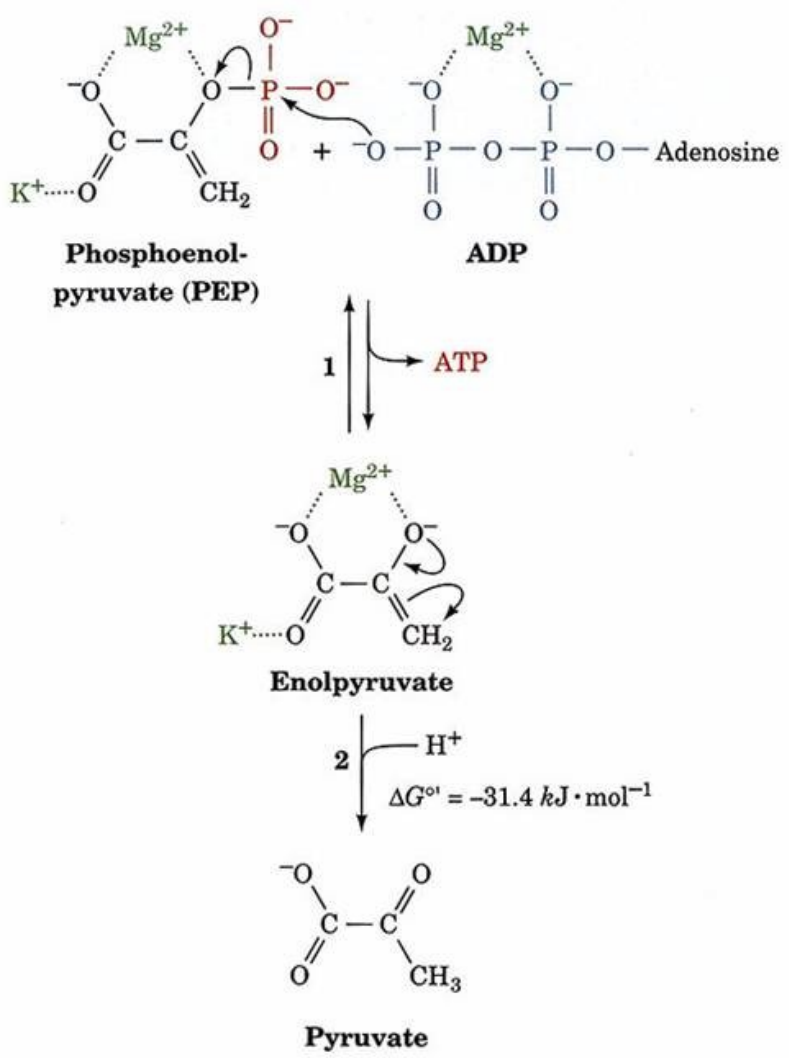


Not a homolog of other kinases in glycolysis

Irreversible under cellular conditions due to large, negative ΔG'

ΔG'° = -31.4 kJ/mol

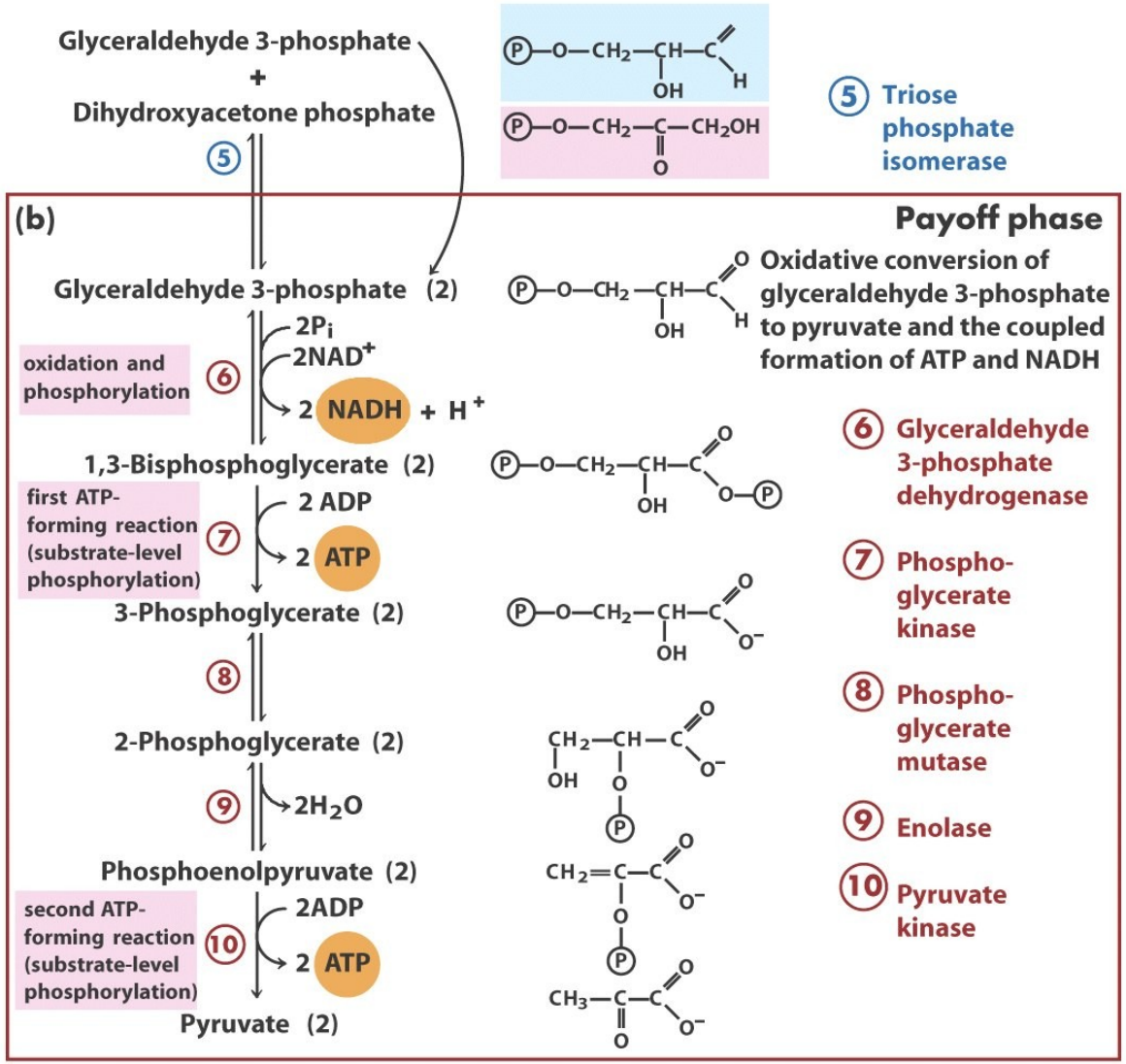
Pyruvate Kinase : Second ATP Generation



Phosphotransfer to ADP

Tautomerization of enolpyruvate to pyruvate.

The Payoff Phase



4 ATP
2 NADH

Overall: glycolysis generates 2 ATP and 2 NADH

Summary of Enzyme Properties

Enzyme	EC Class	Mechanism	Intermediate	$\Delta G'^{o}$	$\Delta G'$	Features
Hexokinase	Transferase	Phosphotransferase ATP → ADP	-	**-17**	-27	Costs ATP
G6P Isomerase	Isomerase	Aldose → Ketose	enol	2	-1	
Phosphofructokinase	Transferase	Phosphotransferase ATP → ADP	-	**-14**	**-26**	Costs ADP
Aldolase	Lyase	Schiffs base	eneamine	23	-6	6C sugar → 2x 3C sugar
Triose Phosphate Isomerase	Isomerase	Ketose → Aldose	enol	8	0	
G3P Dehydrogenase	Oxidoreductase	NAD+ → NADH; SLP	thioester	6	0	1,3-BPG (Substrate level phosphorylation); NADH
Phosphoglycerate Kinase	Transferase	Phosphotransferase ADP → ATP	-	-18	-1	Generates ATP
Phosphoglycerate Mutase	Isomerase	Phosphate migration	2,3-BPG	4	-1	
Enolase	Lyase	Dehydration; Mg2+	enediol	8	-2	PEP
Pyruvate Kinase	Transferase	Phosphotransferase ADP → ATP	-	**-31**	**-14**	a-keto -CO2- ; generates ATP

Reactions with large ΔG' tend to be regulatory targets (Red)