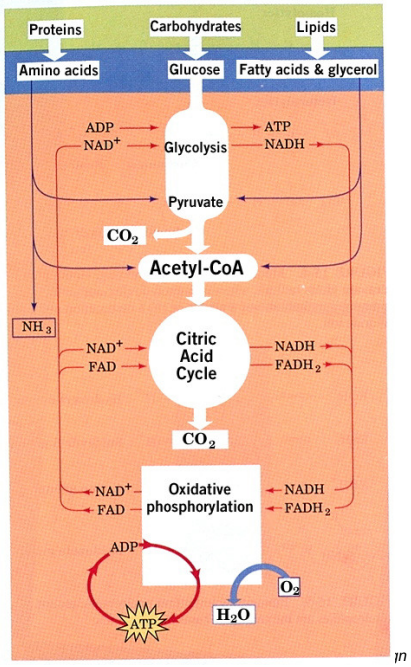
# Glycolysis

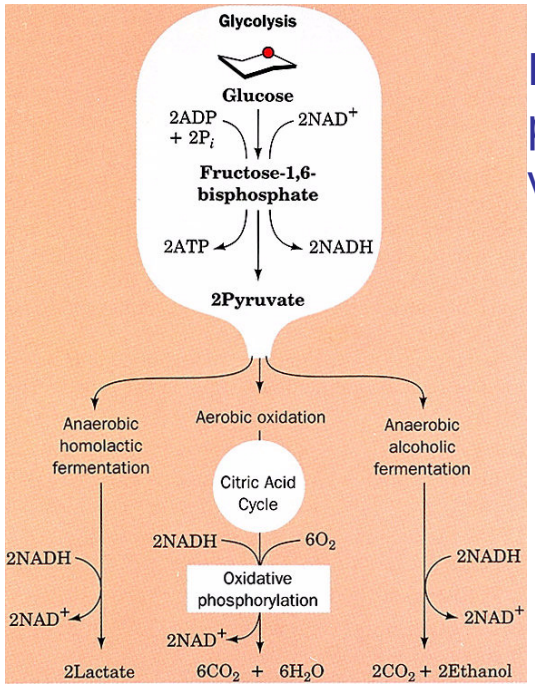
- “Sugar cleavage”
- Glucose is cleaved and oxidized to two molecules of pyruvate (C6 → 2C3)
- The fate of pyruvate depends on whether metabolism is “aerobic” or “anaerobic”




## An overview of aerobic catabolism





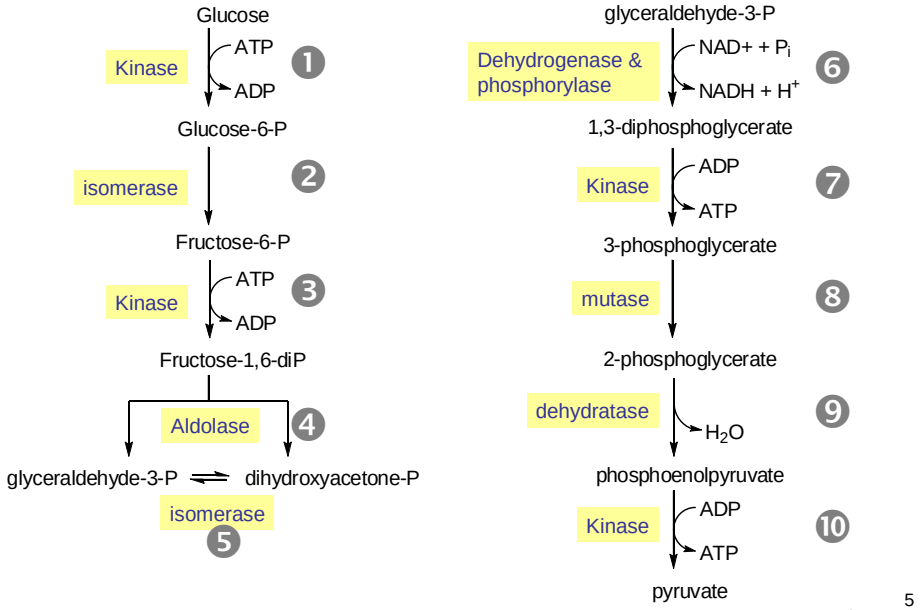

## Fates of pyruvate: aerobic vs, anaerobic




## Glycolysis overview

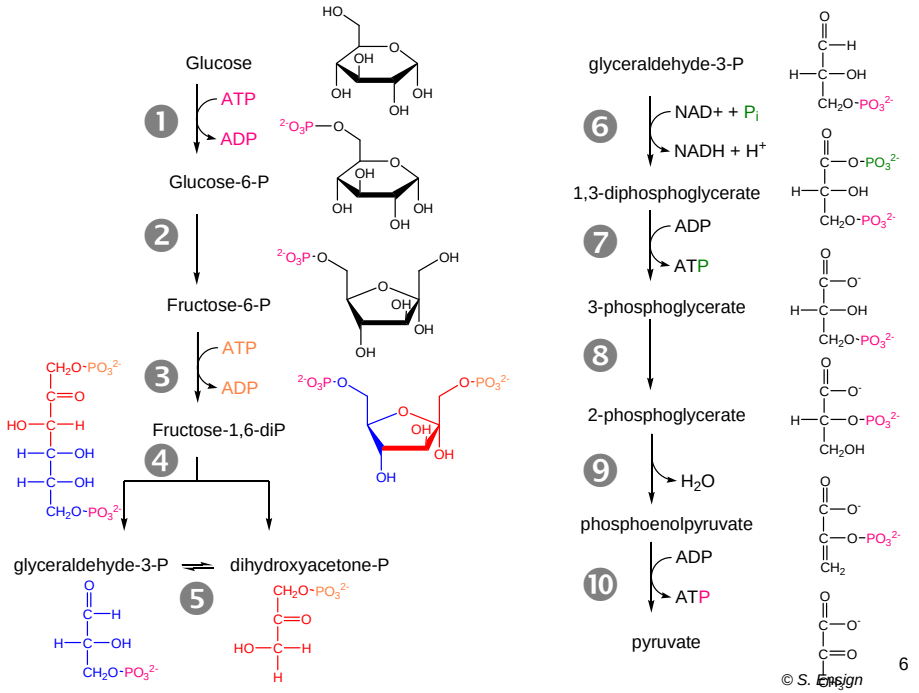
- 10 reactions
- enzymes: kinases, isomerases, aldolase, dehydrogenase, mutase, dehydratase
- The initial five reactions invest 2 ATP in forming two molecules of glyceraldehyde-3-phosphate
- The final five reactions generate 4 ATP from the further catabolism of the two molecules of glyceraldehyde-3-phosphate
- overall reaction:

glucose + 2 $NAD^+$ + 2 ADP + 2$P_i$ → 2 pyruvate + 2 NADH +2$H^+$ +2 ATP +2 $H_2O$







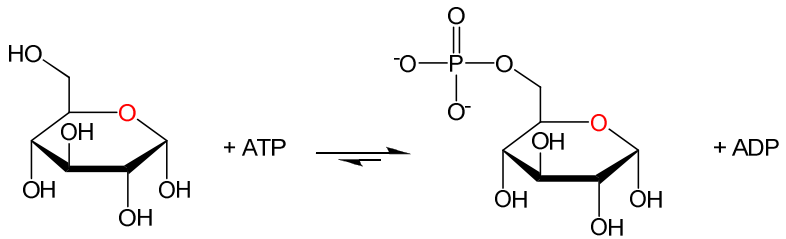








# Reaction 1: Hexokinase

- Phosphoryl group transfer from ATP to C6 hydroxyl of glucose




D-glucose

D-glucose-6-phosphate

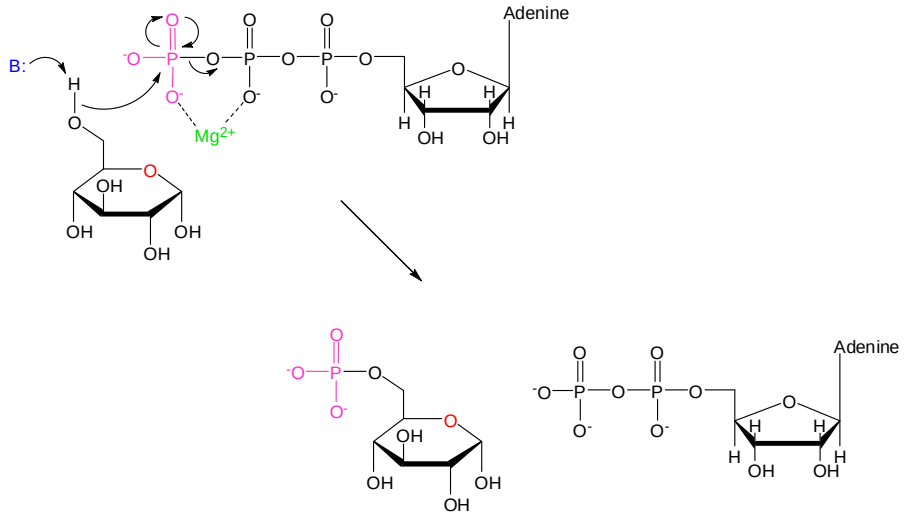
$\Delta G'^{\circ} = -25.1$ kJ/mol

What type of phosphate-containing compound is formed here?



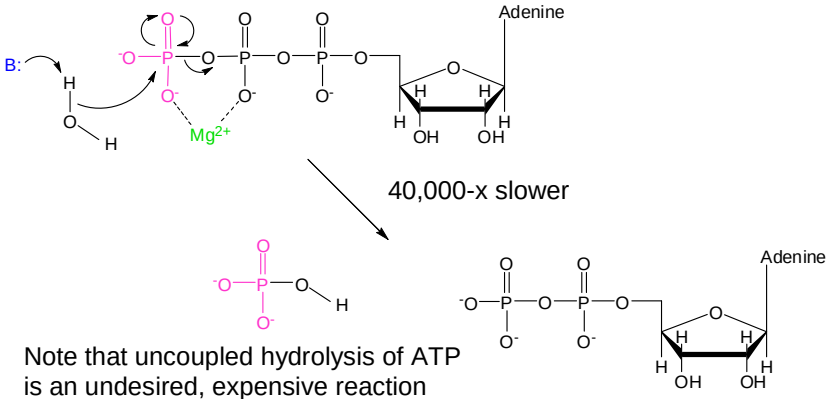


# Mechanism of hexokinase



## Hexokinase is highly specific for using the C6 hydroxyl of D-glucose as the nucleophile for attack on ATP

- transfer of $P_i$ to glucose is 40,000 x greater than to $H_2O$, even though [$H_2O$] is ~10,000-x higher than [glucose] in cells!




Note that uncoupled hydrolysis of ATP is an undesired, expensive reaction

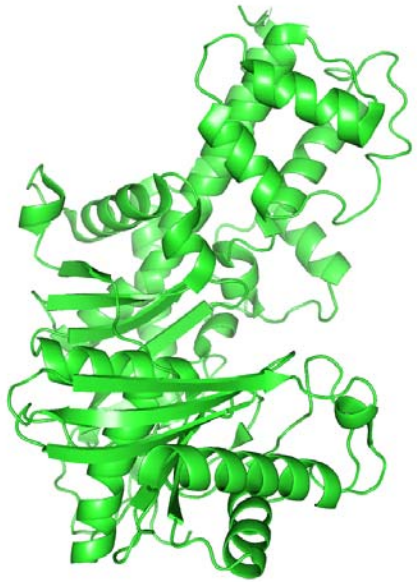



## "Induced fit"

- Binding of substrate induces a conformational change in the enzyme
- In the case of hexokinase, a conformational change induced by glucose binding closes access to the active site and prevents water entry



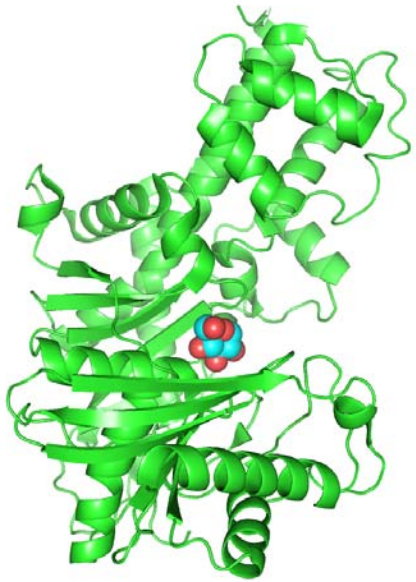

## Open conformation of hexokinase



PDB 1IG8

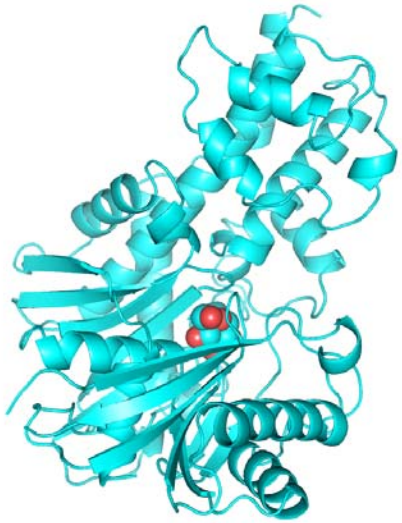



## Open conformation of hexokinase with glucose modeled at active site



PDB entries: open conformation, 1IG8; glucose from closed conformation 1BDG

## Closed conformation of hexokinase with glucose bound at active site

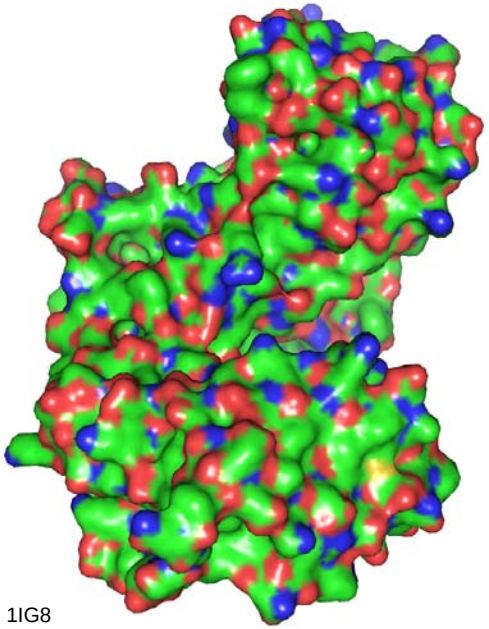


PDB 1BDG





## Open conformation of hexokinase

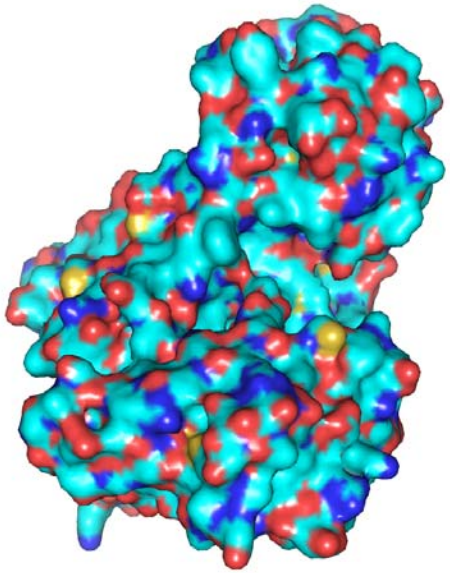


PDB 1IG8





## Closed conformation of hexokinase with glucose bound at active site

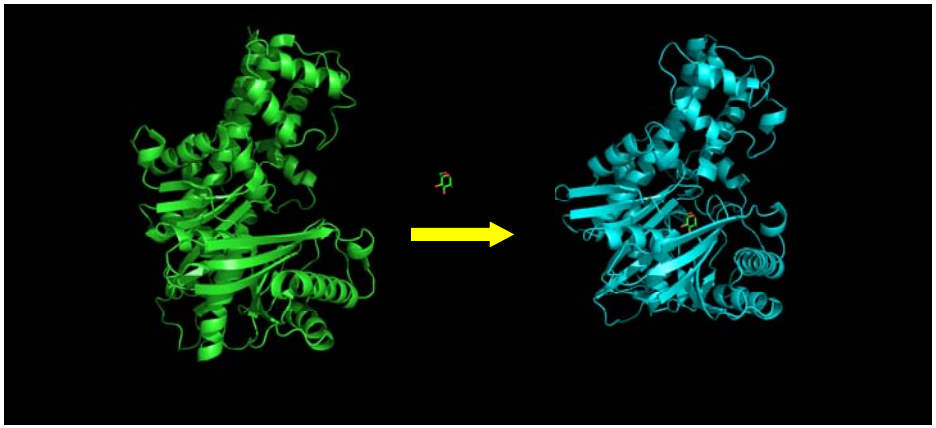


PDB 1BDG





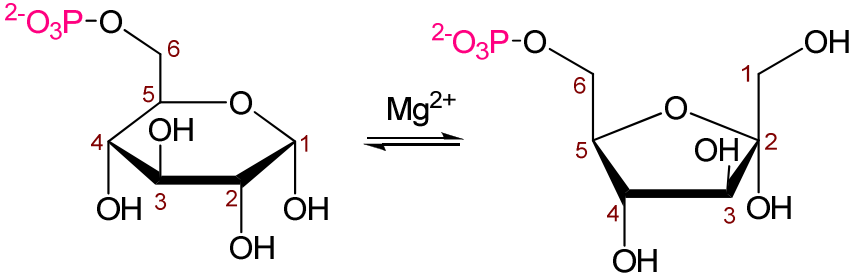
## Note how hexokinase clamps down on it's substrate, preventing water entry into the "activated" active site



PDB entries: open conformation, 1IG8; closed with glucose, 1BDG

# Reaction 2: Phosphoglucoisomerase

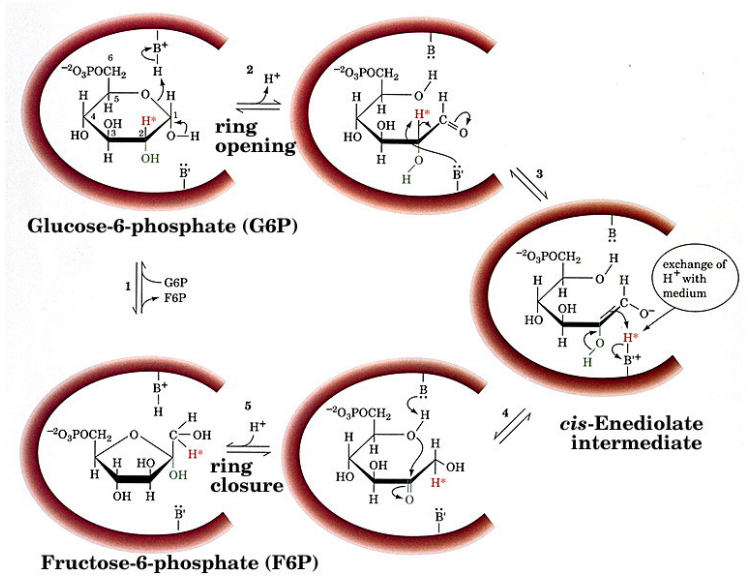
- Isomerization of Glc-6-P to Frc-6-P
- Open ring, isomerize, close ring
- General acid/base chemistry
- A common theme: cis-enediolate intermediate




D-glucose-6-phosphate D-fructose-6-phosphate

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



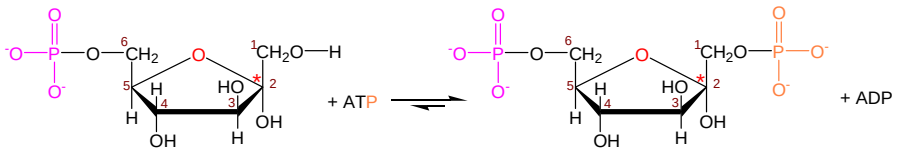







# Reaction 3: Phosphofructokinase

- Phosphoryl group transfer from ATP to C1 hydroxyl of Fructose-6-phosphate



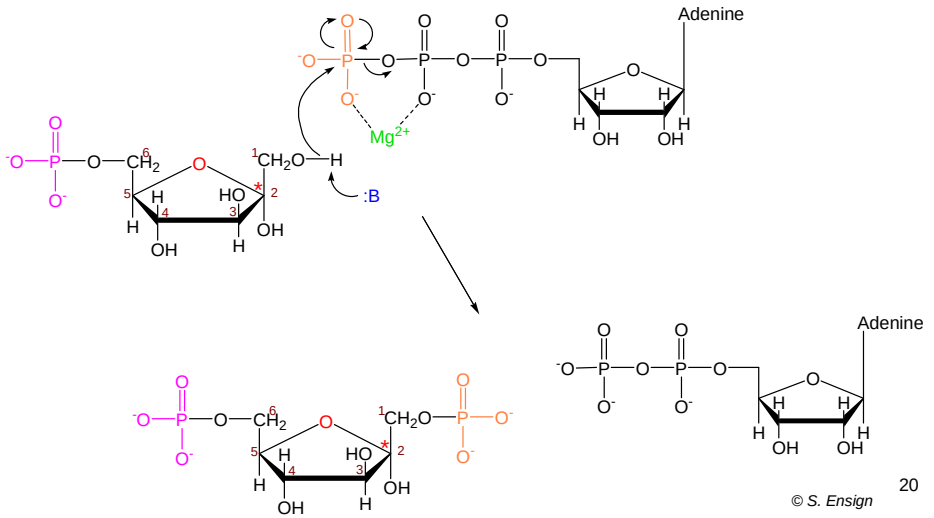

fructose-6-phosphate fructose-1,6-diphosphate

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



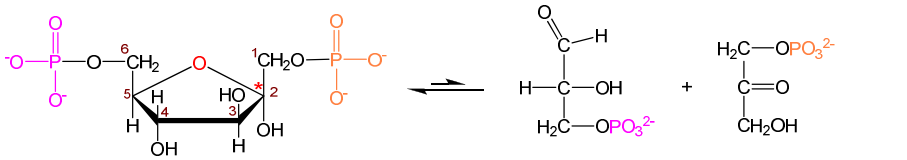

# Reaction 3: Phosphofructokinase

- Phosphoryl group transfer from ATP to C1 hydroxyl of Fructose-6-phosphate



# Reaction 4: Aldolase

- C-C bond cleavage
- "Aldol" cleavage: the reverse of Aldol condensation
- Class I and Class II aldolase: differ in strategy for carbanion stabilization



fructose-1,6-diphosphate | glyceraldehyde-3-phosphate | dihydroxyacetone phosphate

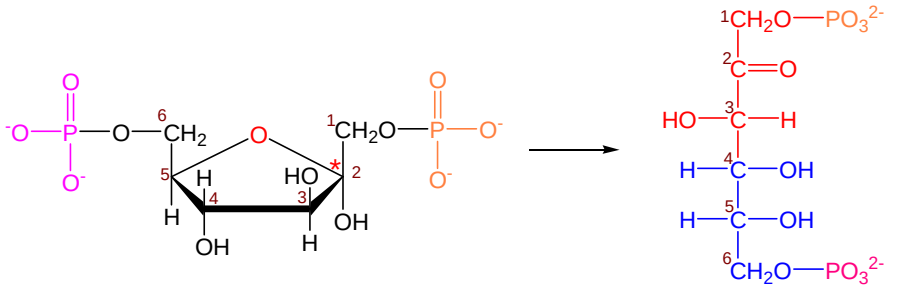
$\Delta G'^{\circ} = +23.8$ kJ/mol





# Aldolase-catalyzed C-C bond cleavage

- The chemistry is easier to visualize when working with the linear Fischer projection
- numbering and color coding help to keep track of which atoms end up where in products of metabolic reactions and pathways

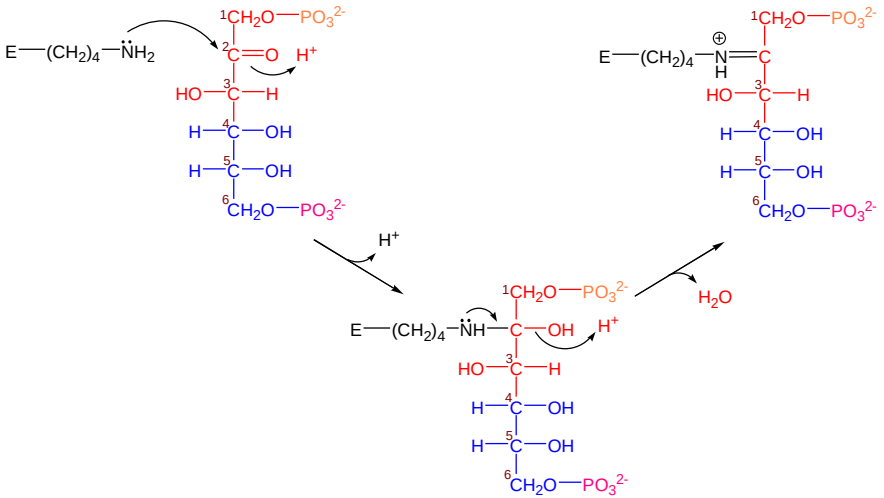


fructofuranose-1,6-diphosphate | fructose-1,6-diphosphate





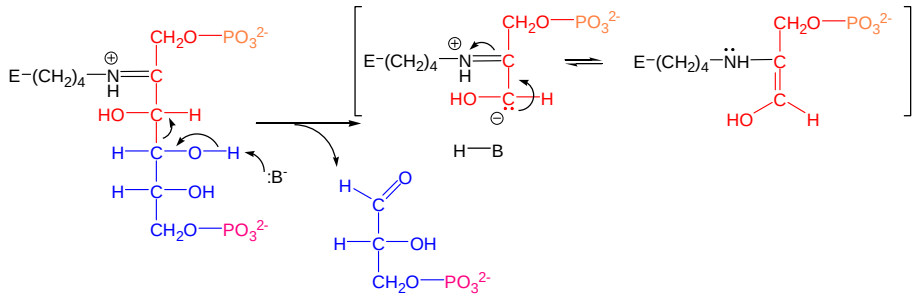
# Class I aldolase mechanism: formation of the Schiff base



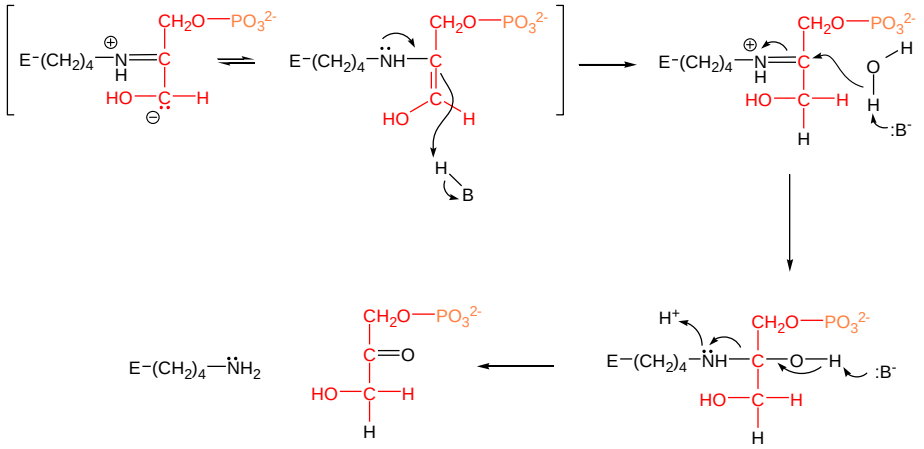




# Class I aldolase mechanism: C-C bond cleavage and generation of the resonance-stabilized carbanion

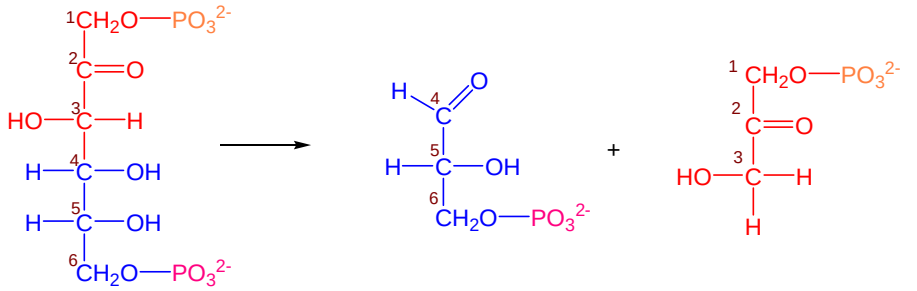


## Class I aldolase mechanism: protonate the carbanion and reverse Schiff base formation to form DHAP





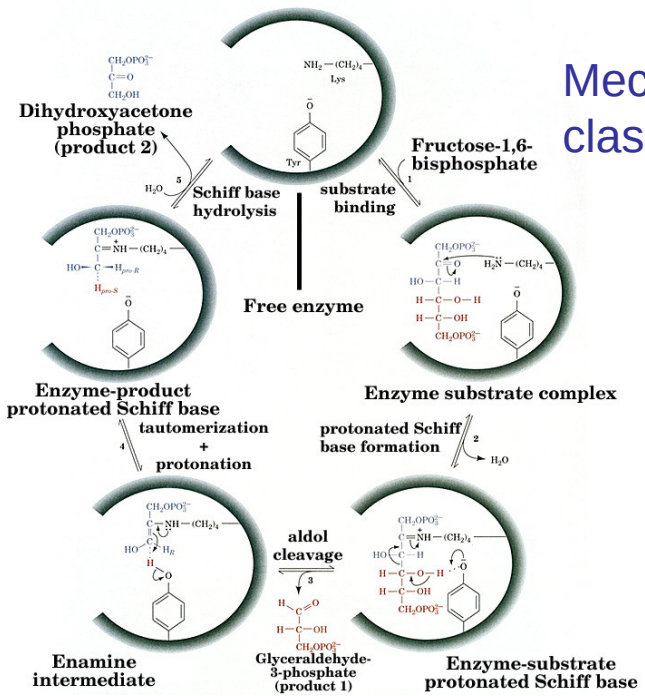

## Aldolase reaction summary







## Mechanism of class I aldolase

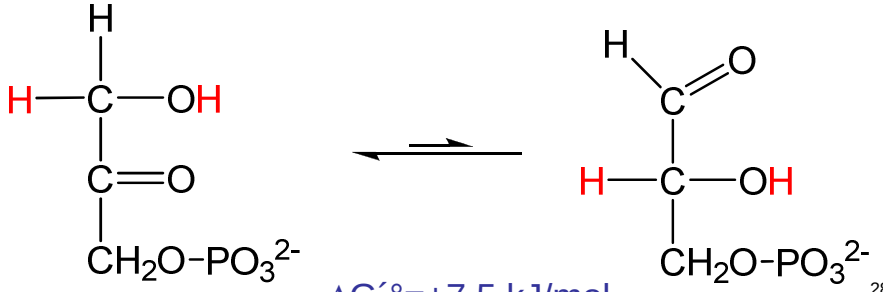






## Reaction 5: Triose phosphate isomerase

- Isomerization of DHAP to G-3-P
- Compare phosphoglucoisomerase
- A "perfect" enzyme: rate is diffusion controlled
- $k_{cat}/K_m = 2 \times 10^8\ M^{-1}{\cdot}s^{-1}$
- $\alpha/\beta$ barrel



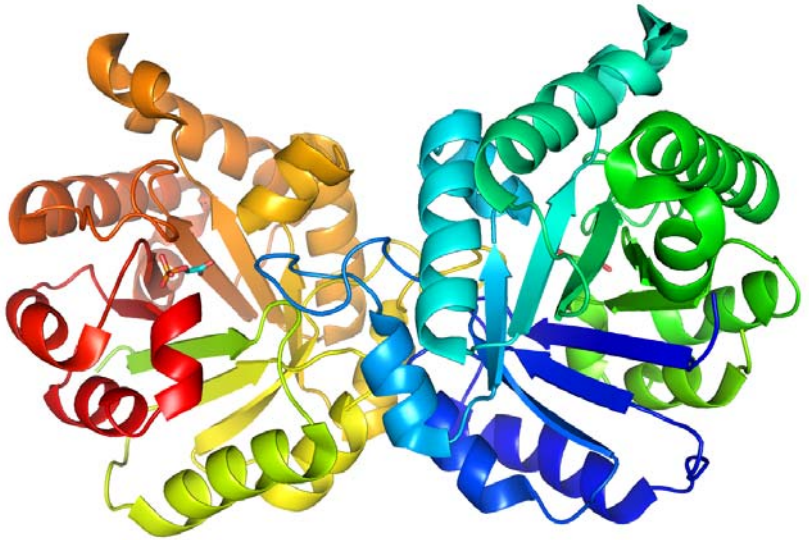
$\Delta G'^{\circ} = +7.5$ kJ/mol

What type of intermediate will triose phosphate isomerase generate during catalysis?

A. stabilized carbanion
B. cis-ene-diolate
C. Schiff base
D. both B and C

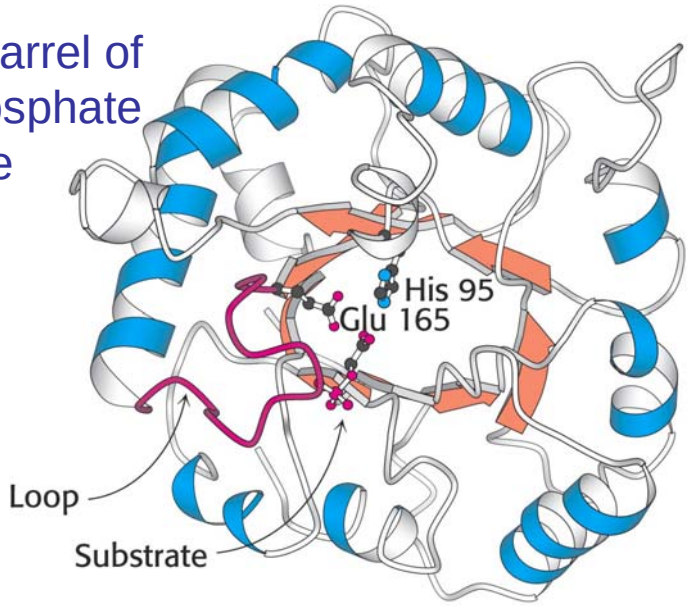


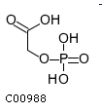

Structure of the TIM dimer with the substrate analog phosphoglycolate bound at the active sites






The α/β barrel of triose phosphate isomerase



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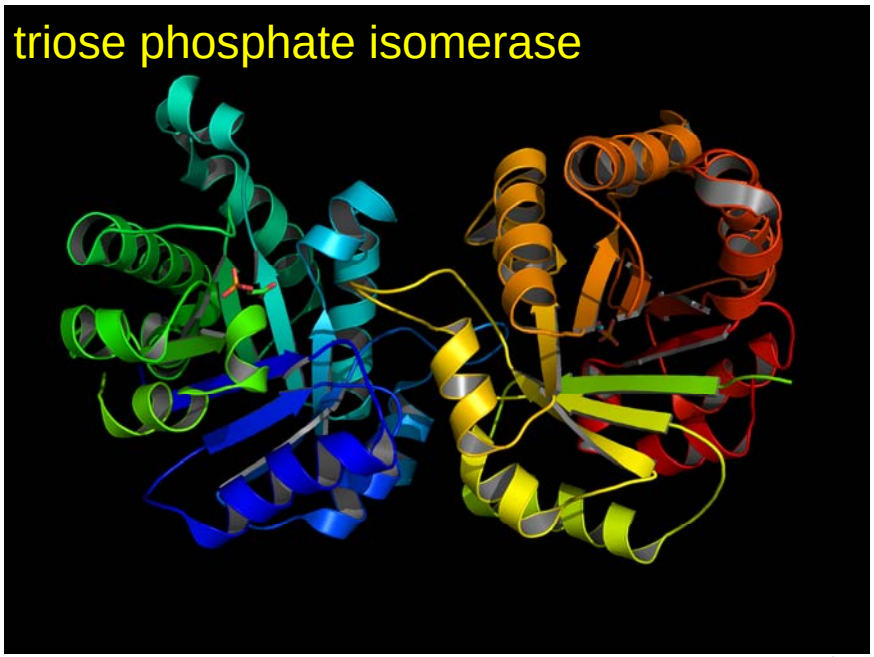
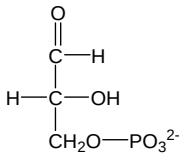
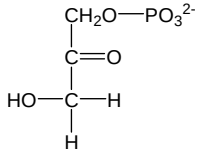


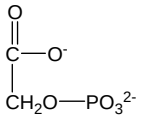
Enzymes are often crystallized with inhibitors (substrate analogs) to provide insights into how the natural substrate binds to the enzyme



glyceraldehyde-3-phosphate



dihydroxyacetone phosphate



phosphoglycolate

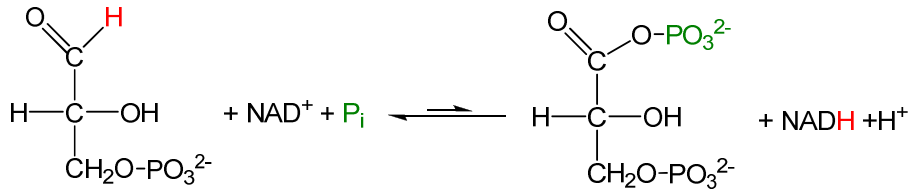


# Reaction 6: Glyceraldehyde-3-phosphate dehydrogenase



# Reaction 6: Glyceraldehyde-3-phosphate dehydrogenase

- Oxidation/reduction together with acyl (or phosphoryl) group transfer
- Covalent acyl enzyme intermediate
- Generates a high energy acyl phosphate



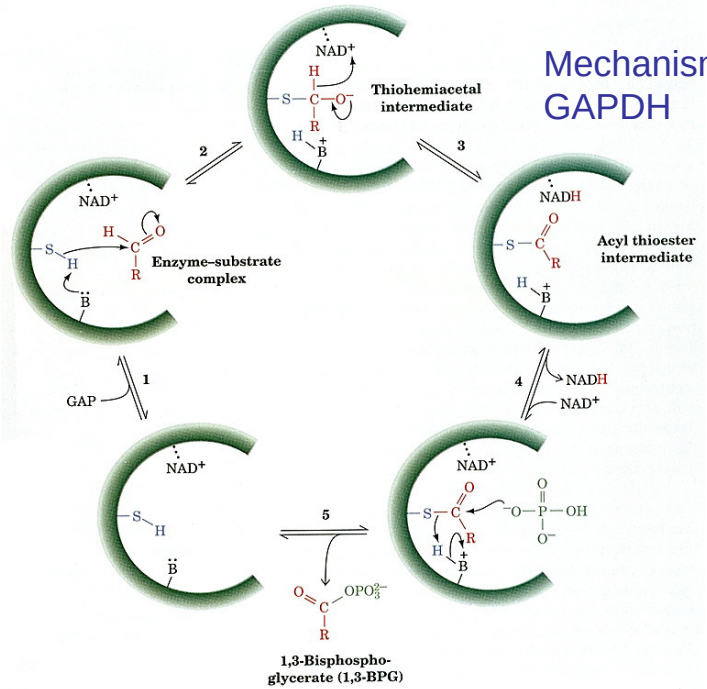

glyceraldehyde-3-phosphate

1,3-diphosphophoglycerate

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

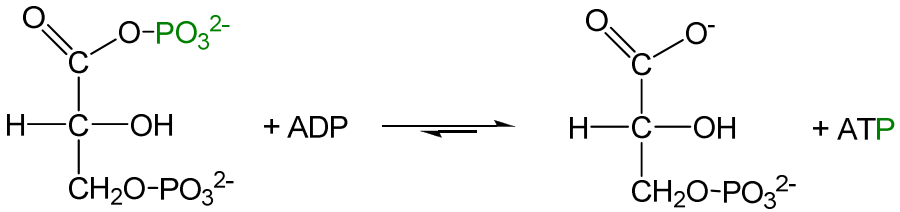


Mechanism of GAPDH



# Reaction 7: phosphoglycerate kinase

- Phosphoryl group transfer from 1,3-DPG to ADP




1,3-diphosphophoglycerate

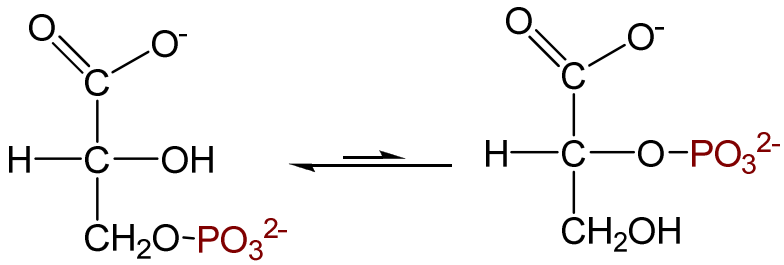
3-phosphophoglycerate

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




# Reaction 8: Phosphoglycerate mutase

- Intramolecular phosphoryl group transfer
- Phosphoenzyme intermediate
- 2,3-DPG intermediate

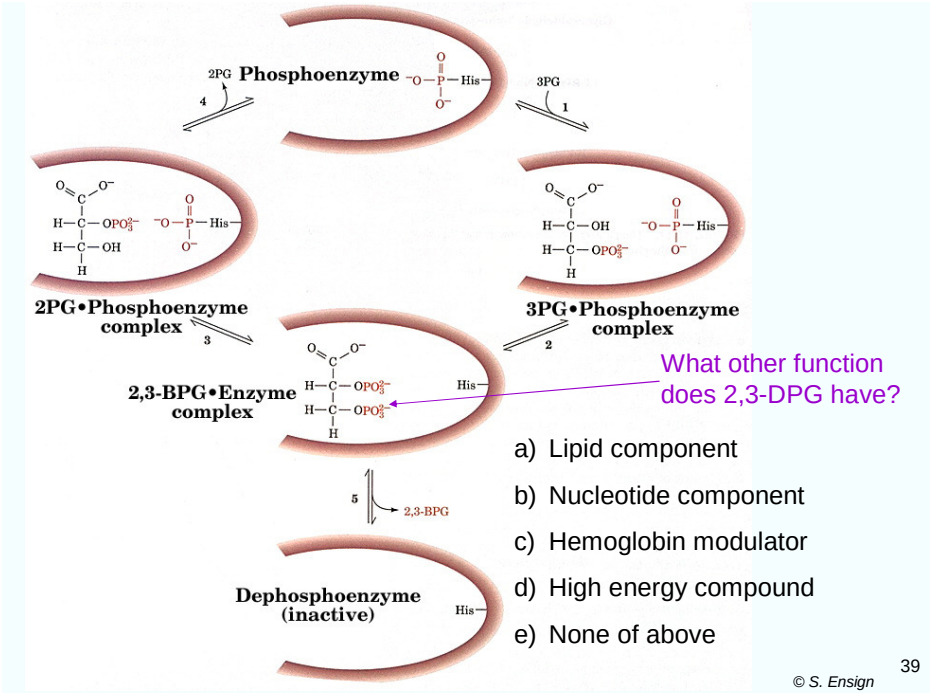


3-phosphophoglycerate

2-phosphophoglycerate

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

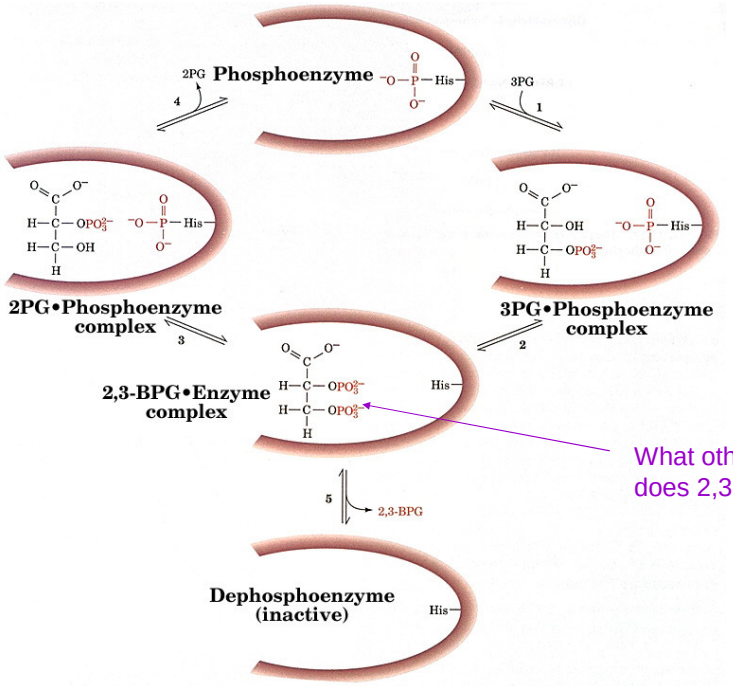



What other function does 2,3-DPG have?

a) Lipid component
b) Nucleotide component
c) Hemoglobin modulator
d) High energy compound
e) None of above

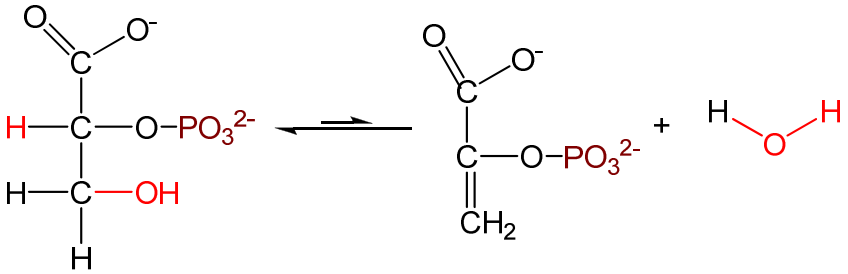



What other function does 2,3-DPG have?

# Reaction 9: Enolase

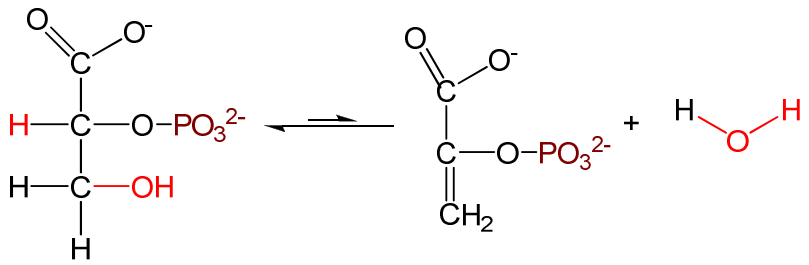
- Dehydration
- General acid/base chemistry



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



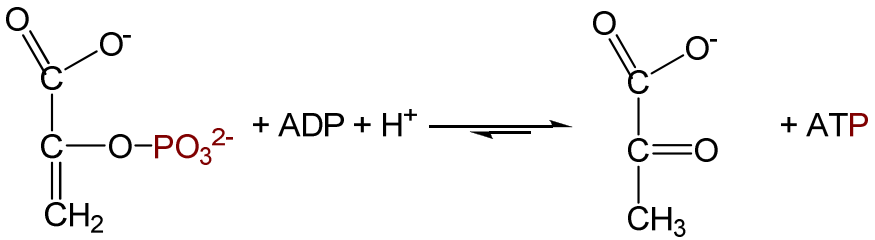

# Enolase mechanism






# Reaction 10: Pyruvate kinase

- Phosphoryl group transfer



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




TABLE 16.3 Reactions of glycolysis

| Step | Reaction | Enzyme | Reaction type | $\Delta G'^{\circ}$ in kcal mol$^{-1}$ (kJ mol$^{-1}$) | $\Delta G$ in kcal mol$^{-1}$ (kJ mol$^{-1}$) |
|---|---|---|---|---|---|
| 1 | Glucose + ATP ⟶ glucose 6-phosphate + ADP + $H^+$ | Hexokinase | Phosphoryl transfer | −4.0 (−16.7) | −8.0 (−33.5) |
| 2 | Glucose 6-phosphate ⟷ fructose 6-phosphate | Phosphoglucose isomerase | Isomerization | +0.4 (+1.7) | −0.6 (−2.5) |
| 3 | Fructose 6-phosphate + ATP ⟶ fructose 1,6-bisphosphate + ADP + $H^+$ | Phosphofructokinase | Phosphoryl transfer | −3.4 (−14.2) | −5.3 (−22.2) |
| 4 | Fructose 1,6-bisphosphate ⟷ dihydroxyacetonephosphate + glyceraldehyde 3-phosphate | Aldolase | Aldol cleavage | +5.7 (+23.8) | −0.3 (−1.3) |
| 5 | Dihydroxyacetone phosphate ⟷ glyceraldehyde 3-phosphate | Triose phosphate isomerase | Isomerization | +1.8 (+7.5) | +0.6 (+2.5) |
| 6 | Glyceraldehyde 3-phosphate + $P_i$ + $NAD^+$ ⟷ 1,3-bisphosphoglycerate + NADH + $H^+$ | Glyceraldehyde 3-phosphate dehydrogenase | Phosphorylation coupled to oxidation | +1.5 (+6.3) | +0.6 (+2.5) |
| 7 | 1,3-Bisphosphoglycerate + ADP ⟷ 3-phosphoglycerate + ATP | Phosphoglycerate kinase | Phosphoryl transfer | −4.5 (−18.8) | +0.3 (+1.3) |
| 8 | 3-Phosphoglycerate ⟷ 2-phosphoglycerate | Phosphoglycerate mutase | Phosphoryl shift | +1.1 (+4.6) | +0.2 (+0.8) |
| 9 | 2-Phosphoglycerate ⟷ phosphoenolpyruvate + $H_2O$ | Enolase | Dehydration | +0.4 (+1.7) | −0.8 (−3.3) |
| 10 | Phosphoenolpyruvate + ADP + $H^+$ ⟶ pyruvate + ATP | Pyruvate kinase | Phosphoryl transfer | −7.5 (−31.4) | −4.0 (−16.7) |

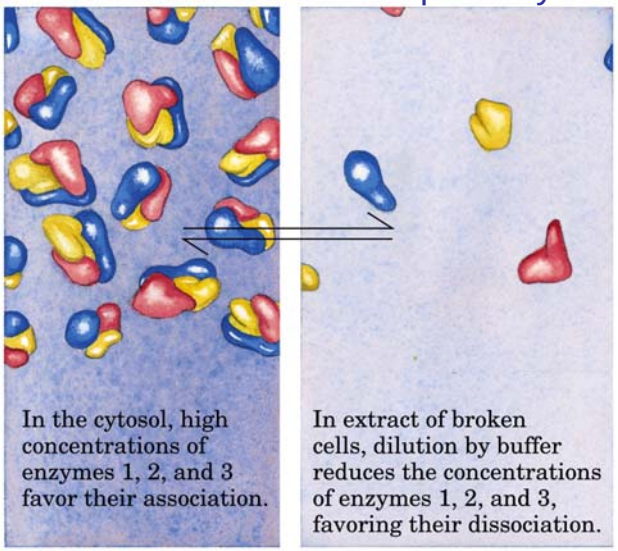
*Note:* $\Delta G$, the actual free-energy change, has been calculated from $\Delta G'^{\circ}$ and known concentrations of reactants under typical physiologic conditions. Glycolysis can proceed only if the $\Delta G$ values of all reactions are negative. The small positive $\Delta G$ values of three of the above reactions indicate that the concentrations of metabolites in vivo in cells undergoing glycolysis are not precisely known.

Net reaction:

glucose + 2 $NAD^+$ + 2 ADP + 2$P_i$ → 2 pyruvate + 2 NADH +2$H^+$ +2 ATP +2 $H_2O$

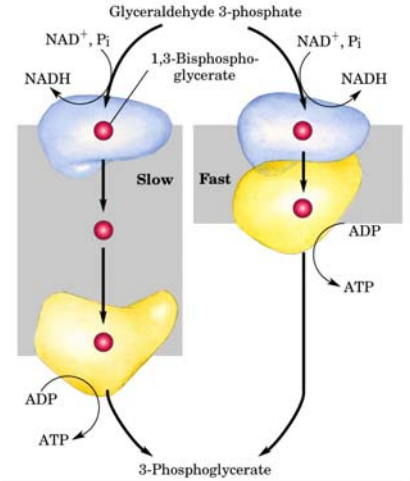
S. Ensign, Chem. 5700

## Multi-protein complexes allow efficient coupling of reactions within a metabolic pathway



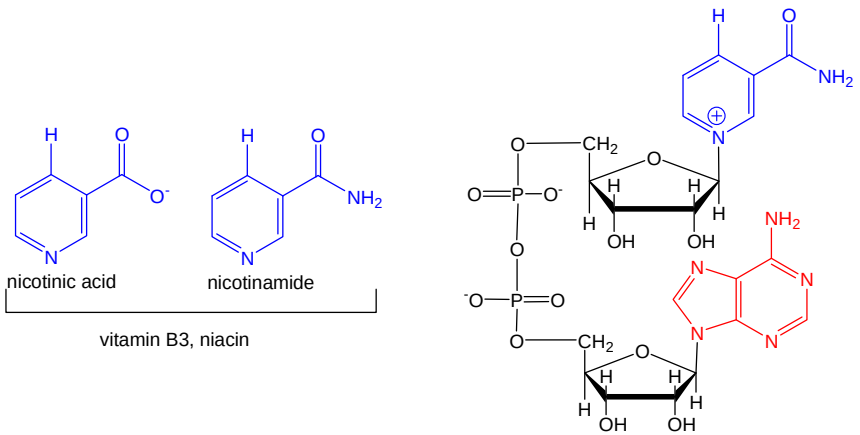








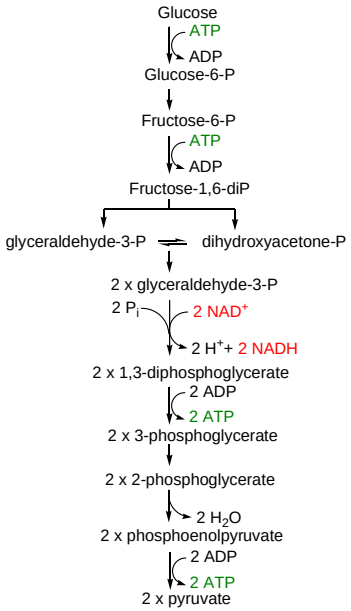




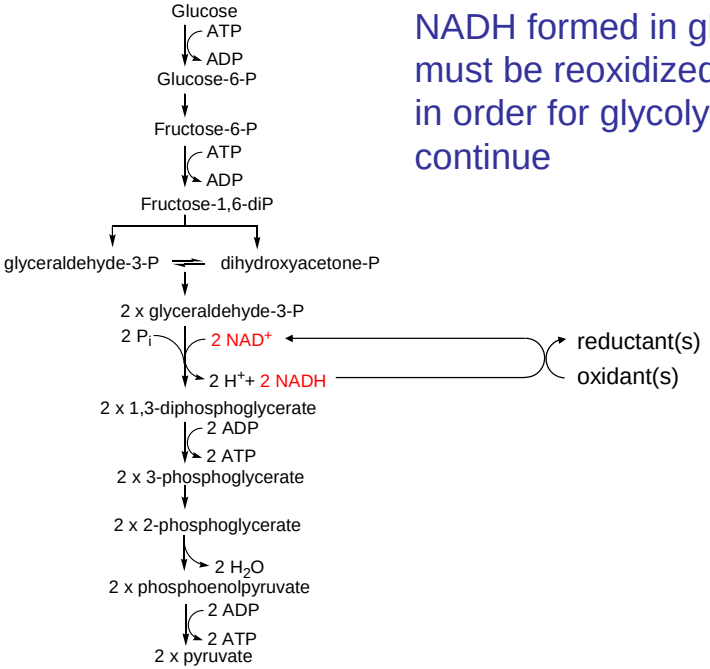




## Summary of glycolysis




glucose + 2 $NAD^+$ + 2 ADP + 2$P_i$ → 2 pyruvate + 2 NADH +2$H^+$ +2 ATP +2 $H_2O$




NADH formed in glycolysis must be reoxidized to $NAD^+$ in order for glycolysis to continue



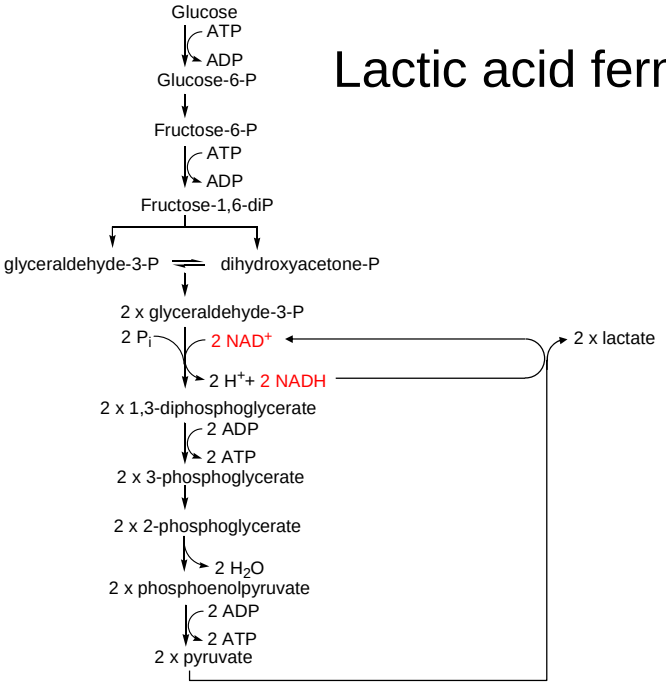

## NADH formed in glycolysis must be reoxidized to $NAD^+$ in order for glycolysis to continue

- Aerobic
  - $O_2$ is the exogenous electron acceptor:

$2\ NADH + O_2 + 2\ H^+ \rightarrow 2\ NAD^+ + 2\ H_2O$

- Anaerobic
  - use exogenous electron acceptors other than $O_2$ ($NO_3^-$, $SO_4^{2-}$, etc)
  - fermentation: use an endogenous electron acceptor, usually an organic molecule; e.g. glucose can be both oxidized and reduced to balance electron flow



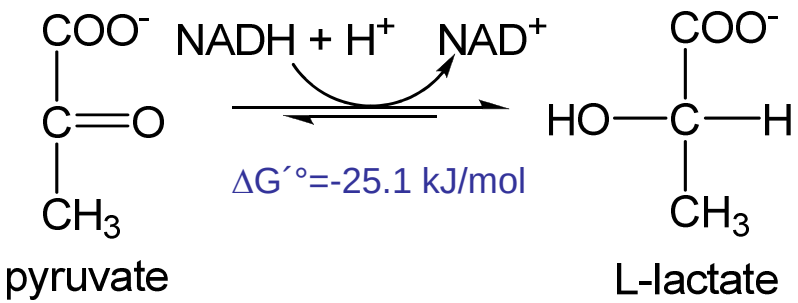




# Lactic acid fermentation



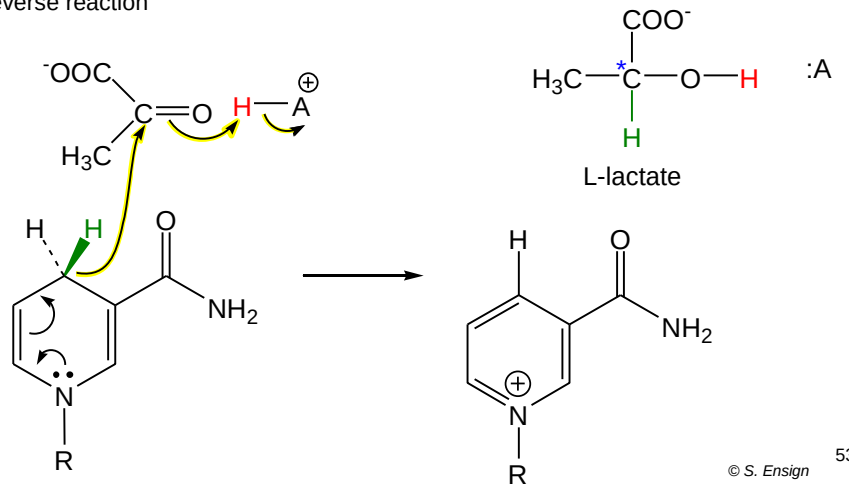

# Lactic acid fermentation

- Regenerate $NAD^+$ under anaerobic conditions



# Lactate dehydrogenase mechanism

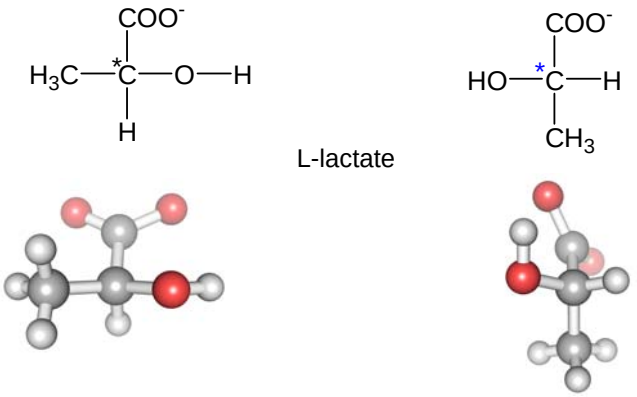
- No Zinc required, just acid/base catalysis
- Note the stereochemical outcome of the reaction
- Dehydrogenases tend to be very stereospecific with regard to both hydride transferred from NADH and the face of the organic acceptor
- Dehydrogenases are in general reversible, so same sterespecificity applies to reverse reaction






# Lactate dehydrogenase mechanism

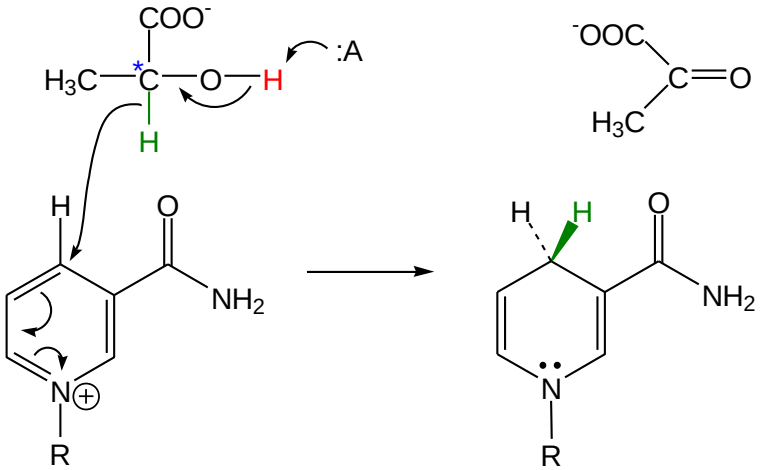
- Note the stereochemical outcome of the reaction





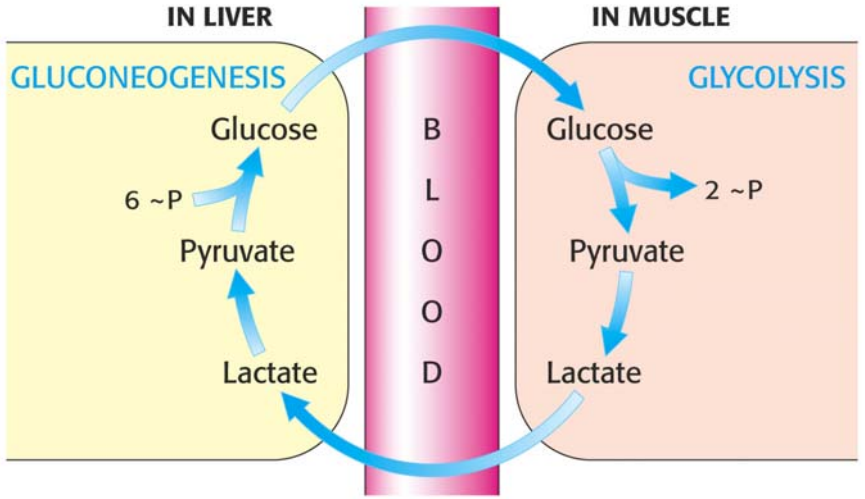

# Lactate dehydrogenase mechanism

- Reverse reaction uses L-lactate as substrate, and transfers hydride to Re face of $NAD^+$



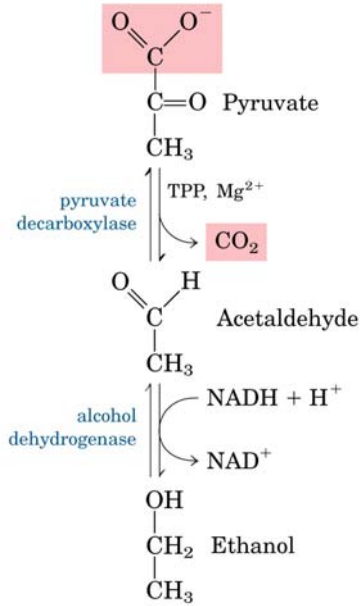



# The Cori Cycle




Anaerobic metabolism is an expensive process in mammals!

Some microorganisms: alcohol fermentation

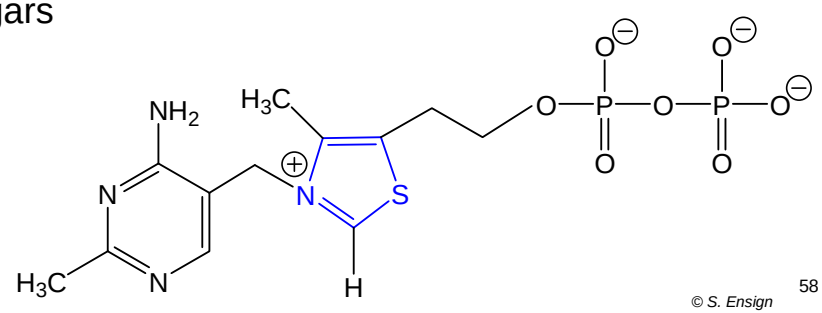






## Thiamine pyrophosphate is a cofactor used in some C-C bond cleavage reactions, including pyruvate decarboxylation

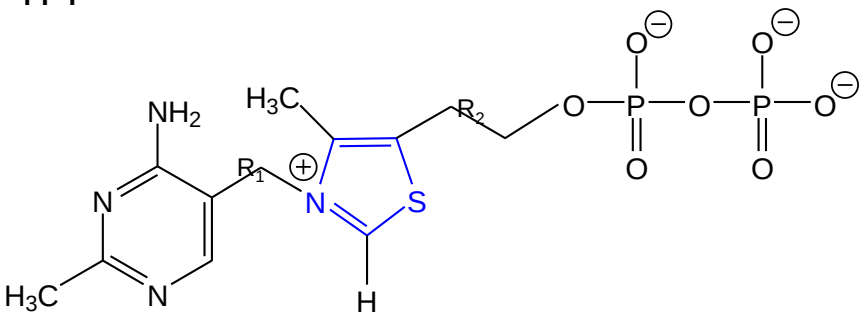
- Pyruvate decarboxylase: “nonoxidative” decarboxylation
- Pyruvate dehydrogenase: “oxidative” decarboxylation
- Transketolase: intermolecular group transfer between sugars






## Formation of TPP carbanion

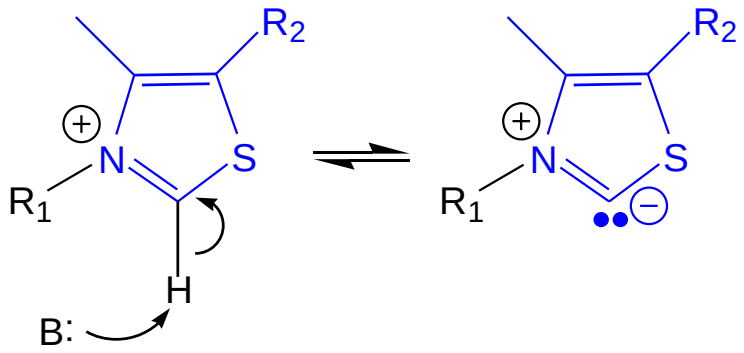
- The thiazolium ring is the “business end” of TPP

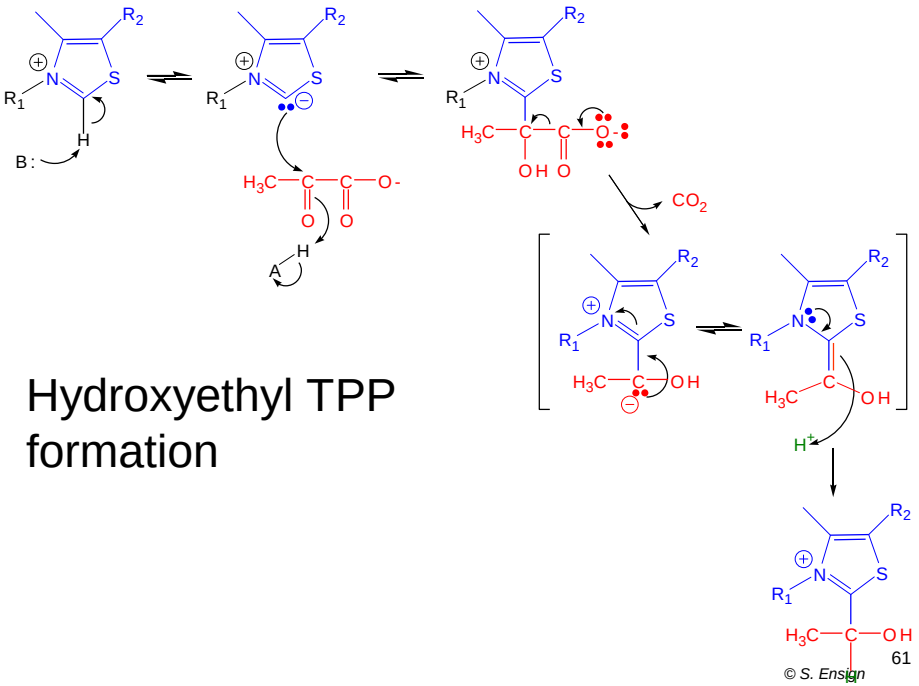





## Formation of TPP carbanion

- The thiazolium ring is the business end of TPP
- C2 of the thiazolium ring is acidic and deprotonates



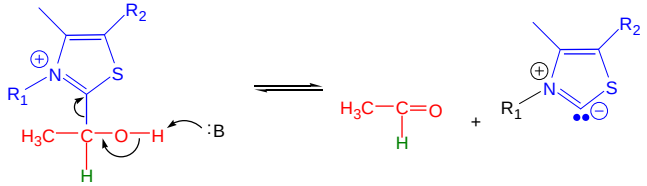


Hydroxyethyl TPP formation





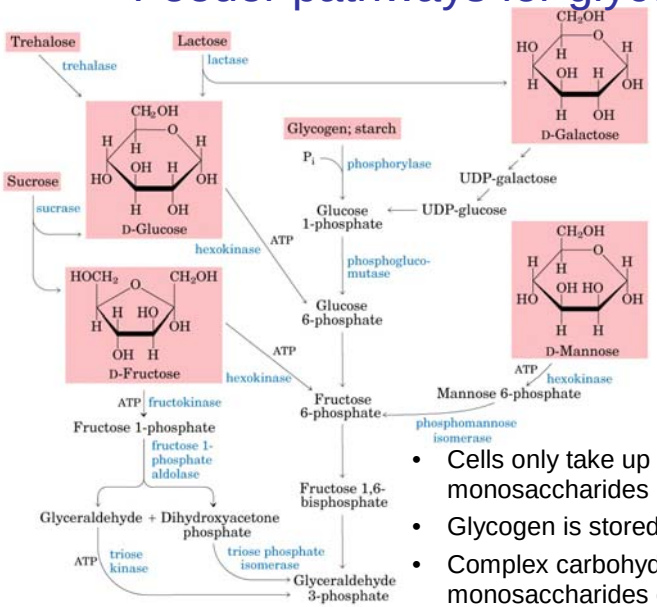
# HETPP breakdown to form acetaldehyde







# Feeder pathways for glycolysis



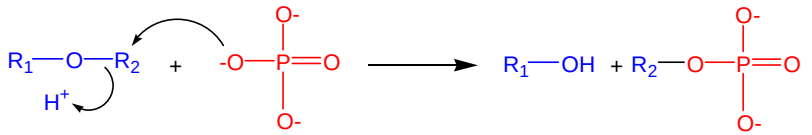

- Cells only take up unphosphorylated monosaccharides
- Glycogen is stored intracellularly
- Complex carbohydrates: hydrolyze to monosaccharides extracellularly



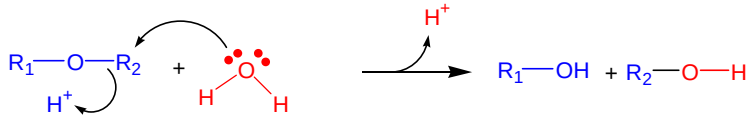


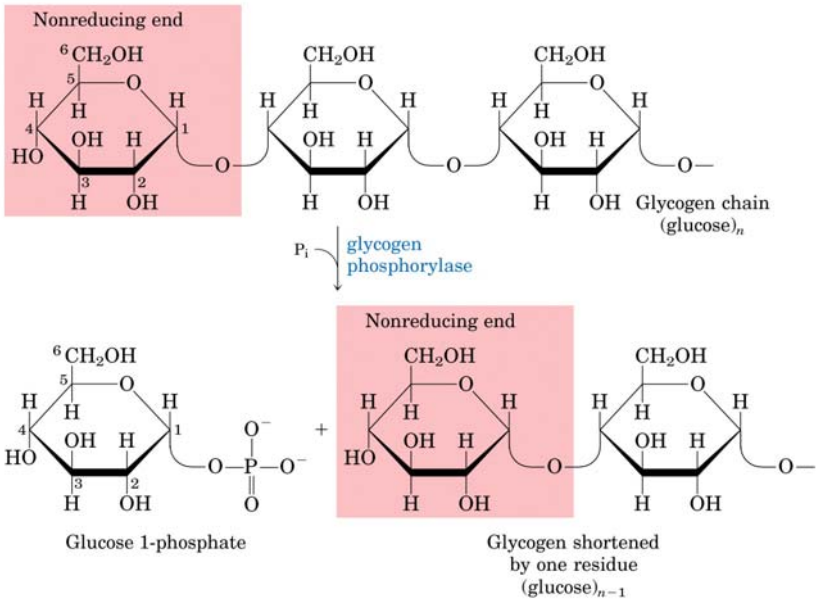
# Phosphorolysis vs. hydrolysis

- Intracellular: phosphorolysis results in direct formation of a phosphorylated organic molecule without having to invest ATP

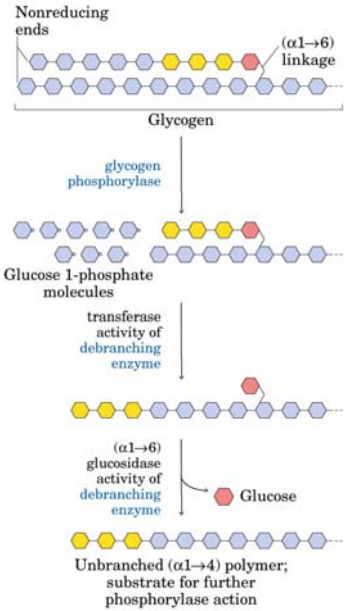


- Extracellular: hydrolysis occurs, because only uncharged monosaccharides are taken up by cells



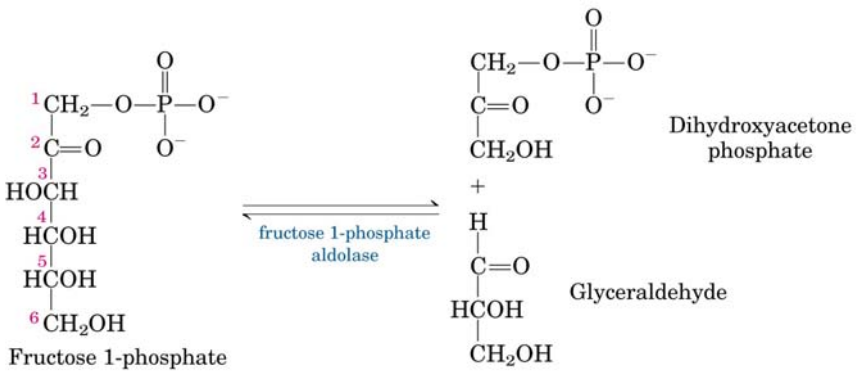




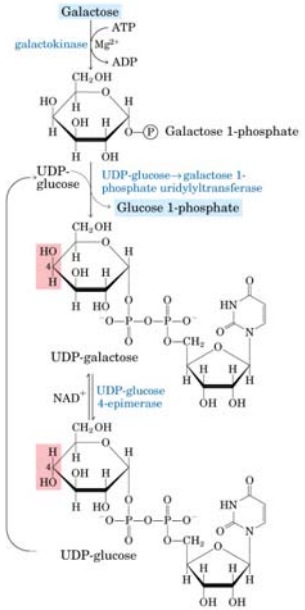








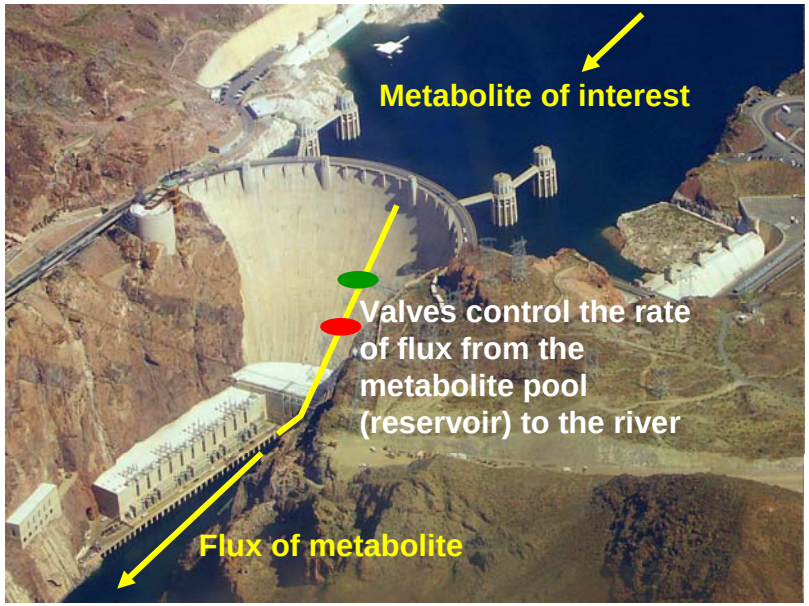




# Regulation of carbohydrate metabolism

- "Flux": the rate of movement of metabolites through pathways
- The rate of "flux" is dependent on the relative activities and levels of the enzymes in a pathway
- "Regulatory enzymes control flux of metabolites
  - "Committed step"
- The same metabolic pathway may be regulated differently in different tissue(s)
  - Muscle: myocytes
  - Liver: hepatocytes




*Credit: http://commons.wikimedia.org/wiki/Image:Hoover_dam_from_air.jpg This image has been (or is hereby) released into the **public domain** by its author, snakefisch at the English Wikipedia project. This applies worldwide.*




Review….

## Regulatory enzymes and metabolic pathways

- Exhibit increased or decreased catalytic activity in response to certain signal(s)
- Usually catalyze the "committed step" in a pathway
- Often modulated by allostery
- "Product" inhibition
- "Feedback" inhibition: the concentration of a *pathway product* controls the activity of an enzyme near the beginning of the pathway

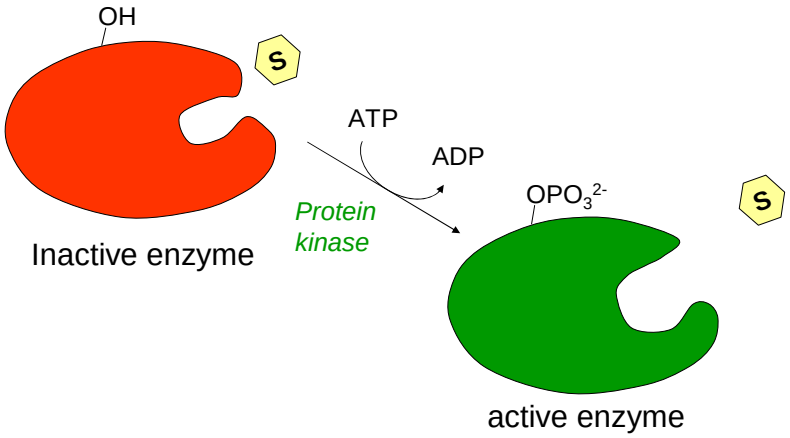



## Ways to regulate enzyme activity

- Level of expression (amount of enzyme produced)
- "Zymogen" activation
- Protein turnover (degradation)
- Covalent modification: on/off switch for an enzyme
- Allosteric effectors: "Attenuated" expression: increase or decrease activity over a broad range of enzyme through binding of "modulators"
- Possible hormonal control of above processes

## Enzyme regulation by reversible phosphorylation/dephosphorylation

Scenario 1: activation by phosphorylation

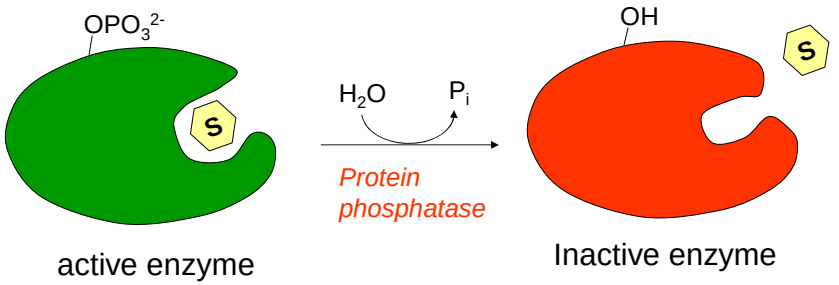






## Enzyme regulation by reversible phosphorylation/dephosphorylation

Scenario 1 (cont.): inactivation by dephosphorylation

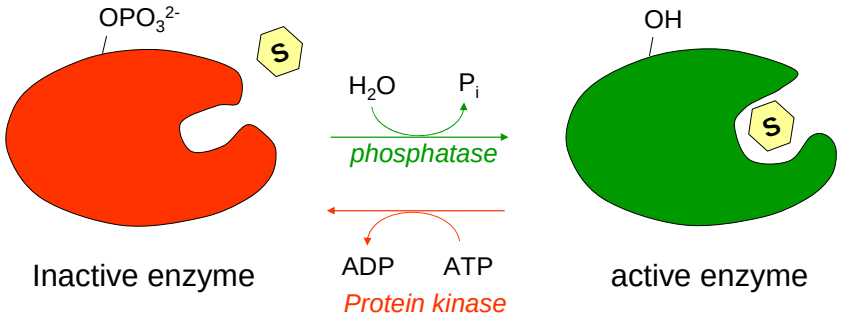






## Enzyme regulation by reversible phosphorylation/dephosphorylation

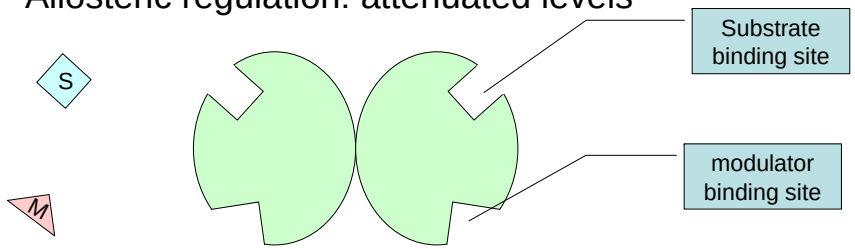
Scenario 2: activation by dephosphorylation and inactivation by phosphorylation







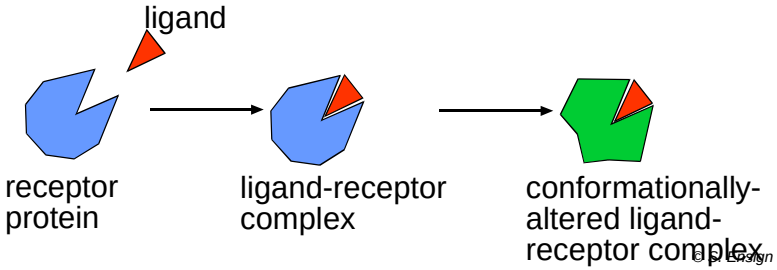
Allosteric regulation: attenuated levels




**Recall that binding of modulators can increase or decrease enzyme activity by inducing conformational changes affecting substrate binding**

## Review of hormone function

- Produced in one tissue, carried in blood stream to target tissue (intercellular)
- Bind to “receptor” proteins
- Binding to the receptor protein triggers a specific change: gene expression, metabolism, etc.






## Glycogen breakdown and glycolysis provide excellent model systems for studying metabolic regulatory mechanisms



## Regulation of Glycolysis

- Pre-glucose (e.g. glucose uptake, glycogen breakdown)
- Post-glucose (glycolytic enzymes)
- Tissue dependence (liver vs. muscle)



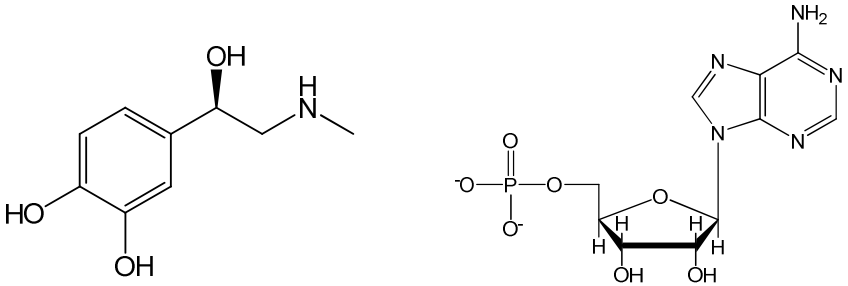
## Pre-glucose: regulation of glycogen phosphorylase

$glycogen_{(n)} + P_i \rightarrow glycogen_{(n-1)} + glucose\text{-}1\text{-}phosphate$

- Under both hormonal and allosteric control
- Hormonal and allosteric control are different for muscle and liver GP
- Hormonal control involves covalent modification of GP to activate the enzyme
  - Phosphorylation by a kinase
  - Dephosphorylation by a phosphorylase

# Control of glycogen phosphorylase in muscle

- Hormonal control: epinephrine (adenaline)
- Allosteric control: AMP






# Adrenaline-stimulated glycogen breakdown

- Epinephrine is released from the adrenal gland in response to environmental “stressors”
- EP travels through the blood stream to target tissue
- EP binds to an extracellular binding site on a transmembrane receptor protein
- EP binding initiates a “cascade” of reactions resulting in glycogen breakdown to glucose

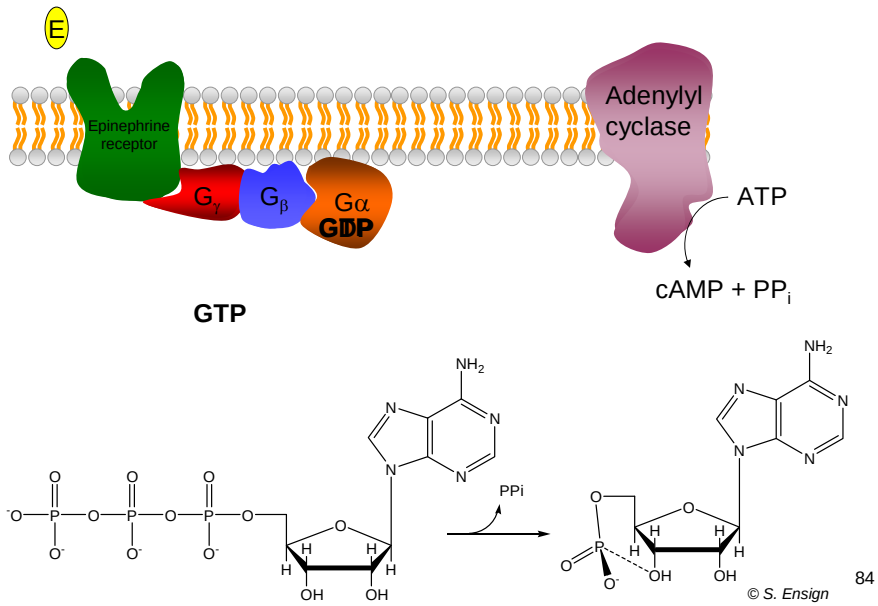



# EP binding initiates a “cascade” of reactions resulting in glycogen breakdown to glucose

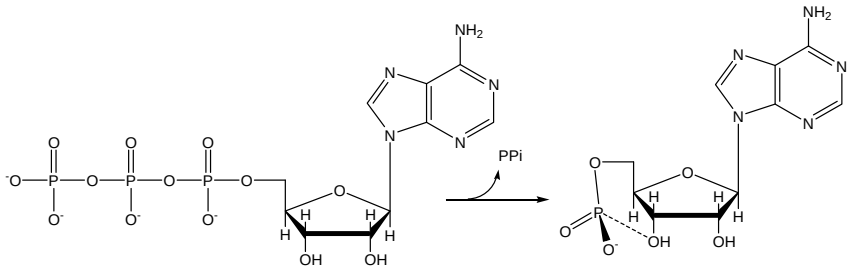
- A “G protein” binds adenylyl cyclase, activating it
- activated adenylyl cyclase produces 3’,5’-cAMP from ATP
- cAMP activates “protein kinase A” by serving as an allosteric effector
- Allosterically-activated protein kinase A transfers a $P_i$ from ATP to phosphorylase kinase, thereby activating it
- Phosphorylated phosphorylase kinase transfers a $P_i$ from ATP to glycogen phosphorylase, thereby activating it
- Phosphorylated glycogen phosphorylase is now active to catalyze the phosphorolysis of glycogen to glucose-1-phosphate



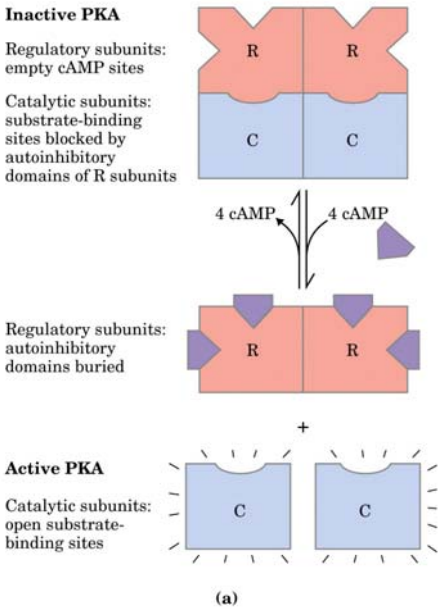

## Epinephrine activation of adenylyl kinase in muscle



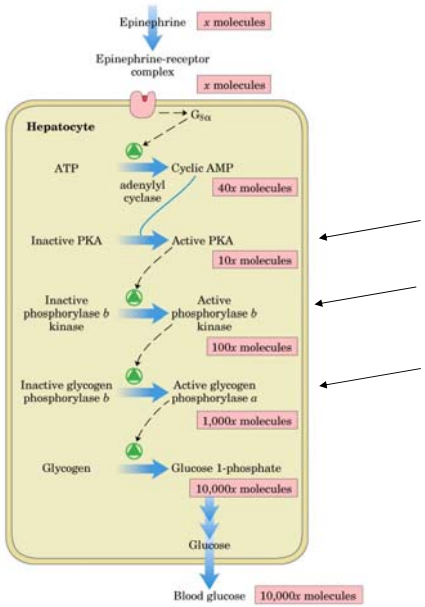
# cAMP is a positive heterotropic effector of protein kinase A







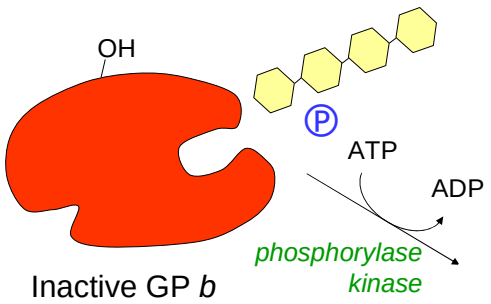

Allosteric activation by cAMP

Activation by phosphorylation, catalyzed by protein kinase A

Activation by phosphorylation, catalyzed by phosphorylase b



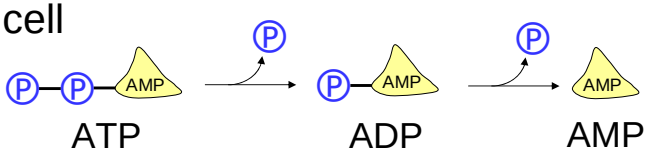
# Activation of glycogen phosphoylase by phosphorylation




Note: the enzyme glycogen phosphorylase regulated in this manner is a dimer; this simple cartoon model shows a monomeric unit for simplicity

# Allosteric control of muscle GP

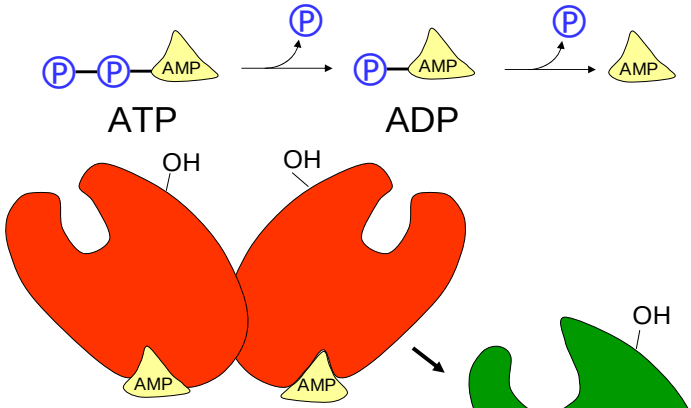
- AMP is a positive heterotropic effector
- High [AMP] is a signal of poor energy status in the cell

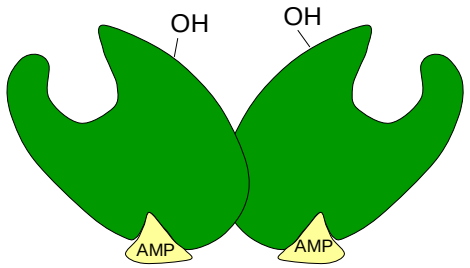



- Activating GP *b* by AMP "buys time", allowing glycogen breakdown until the slower hormonal cascade can complete the conversion of GP *b* to GP *a*



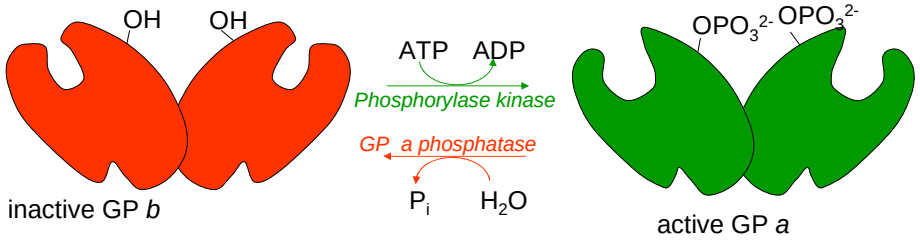

# Activation of GP *b* by AMP

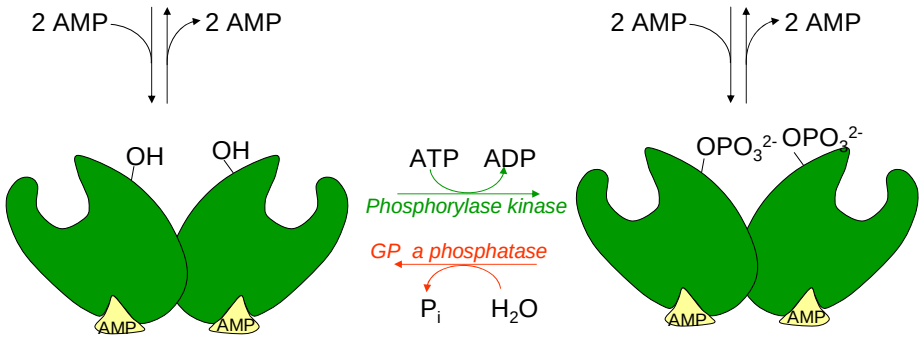






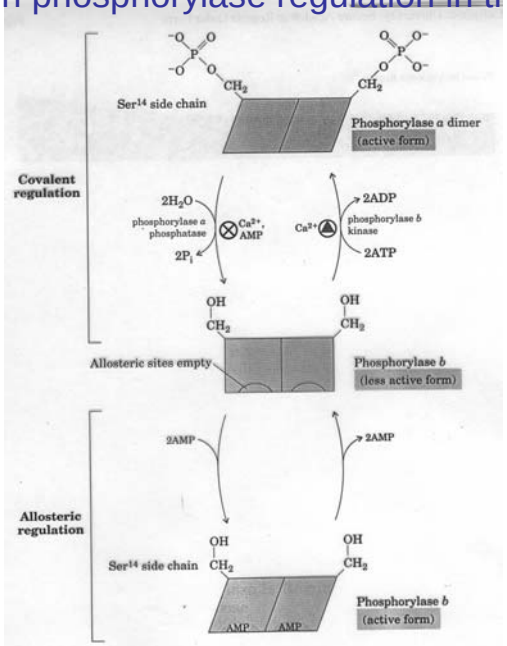






## Glycogen phosphorylase regulation in the muscle



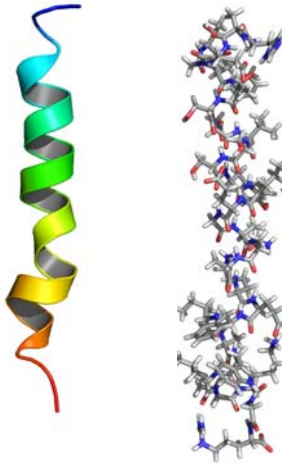
## Control of glycogen phosphorylase in liver

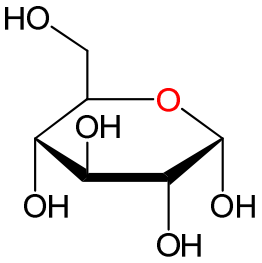
- The liver is used as a reservoir of glucose to be exported to other tissue (contrast muscle)
- When blood glucose levels drop, GP breaks down glycogen and sends glucose to other tissues




## Control of glycogen phosphorylase in liver

- Hormonal control: glucagon
- Allosteric control: glucose

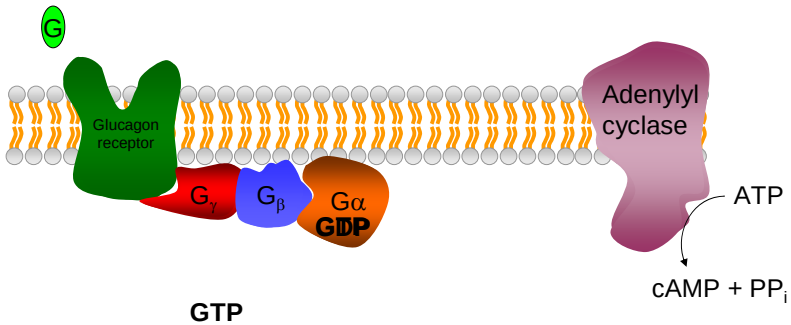




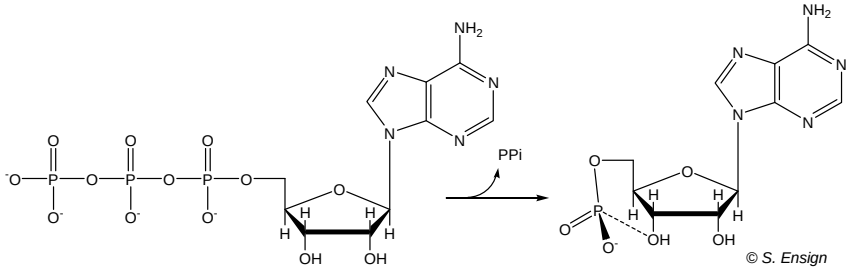




## Glucagon activation of adenylyl kinase in liver



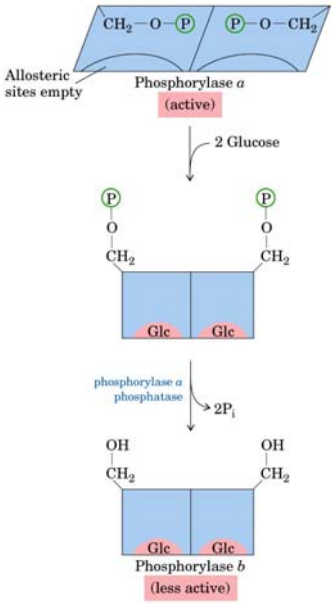

**GTP**







The same reaction cascade activates GP in liver as in muscle, with the exception that glucagon rather than epinephrine is the stimulating hormone



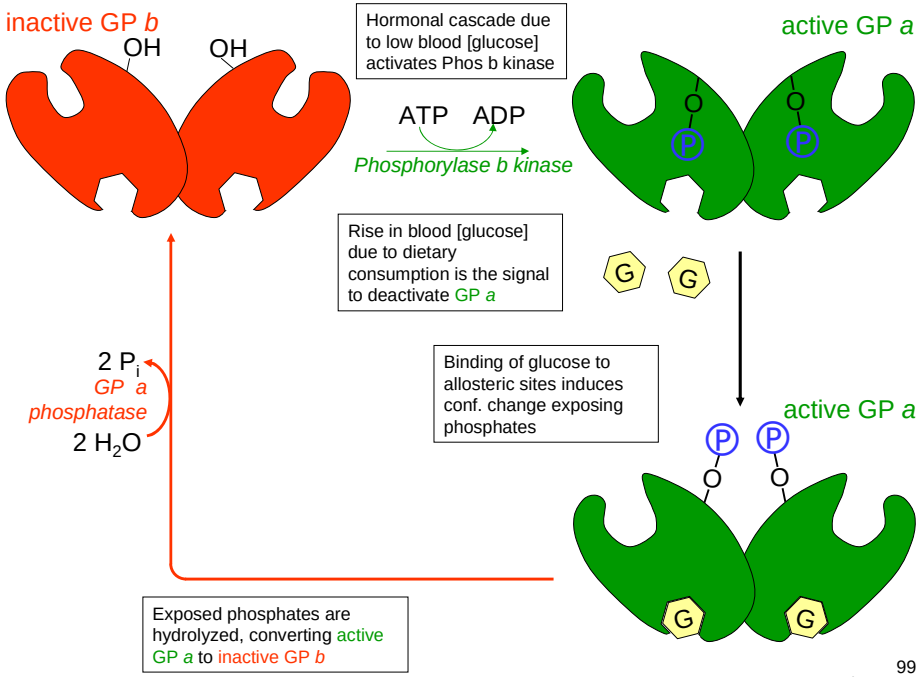

## Liver Glycogen phosphorylase *a* is a glucose level sensor




## Allosteric control of liver GP

- Glucose is a negative heterotropic effector
- High [glucose] is a signal that liver cells no longer need to break down glycogen, and should instead commit to synthesizing new glycogen from free glucose
- Binding of glucose to an allosteric site on GP promotes dephosphorylation of GP *a* by exposing the phosphate groups for hydrolysis by phosphorylase *a* phophatase





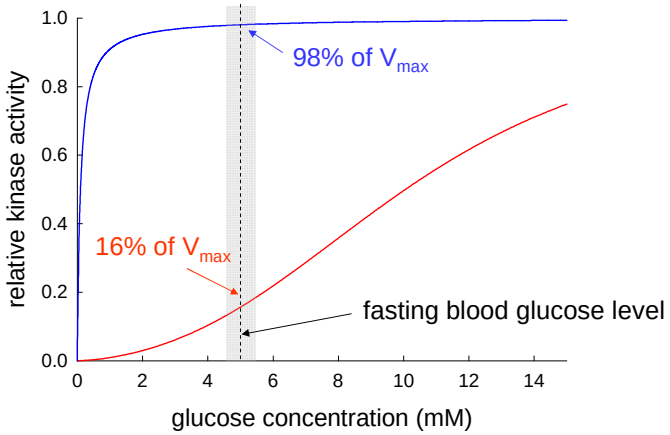


## Regulation of Glycolysis

- Pre-glucose (e.g. glucose uptake, glycogen breakdown)
- Post-glucose (glycolytic enzymes)
- Tissue dependence (liver vs. muscle)

# Isozymes of hexokinase

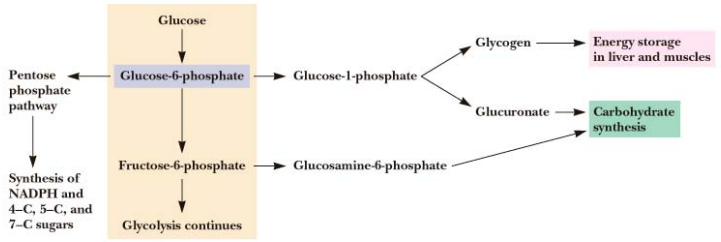
- Hexokinase,muscle: $K_m$ = 0.1 mM, hyperbolic
- Glucokinase,liver: $K_m$ = 10 mM, sigmoidal







# Glucose-6-phosphate is used for other purposes than just glycolysis (still in the plane)



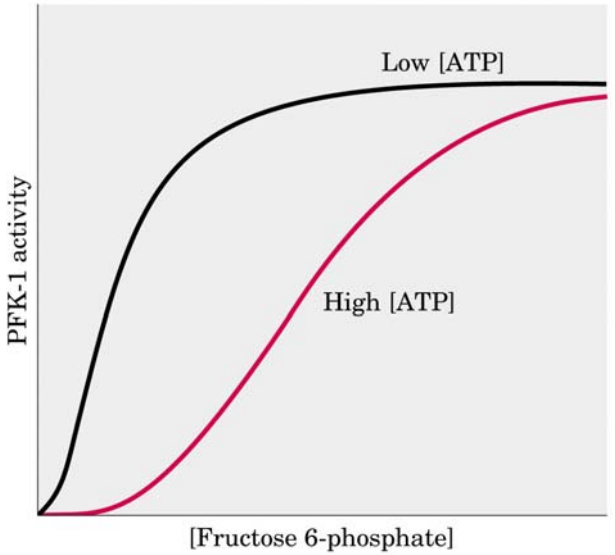




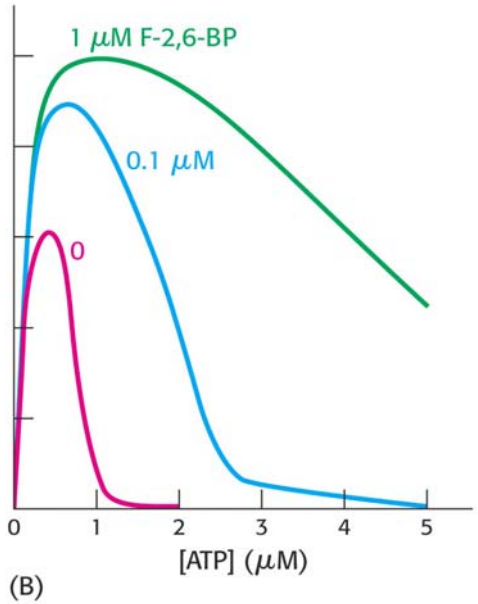
# Phosphofructokinase is the key regulatory enzyme of glycolysis

- ATP: substrate AND negative allosteric regulator ⊗
- ADP and AMP: positive allosteric regulators ▲
- Citrate: negative allosteric regulator ⊗
- Fructose-2,6-diphosphate: positive allosteric regulator ▲



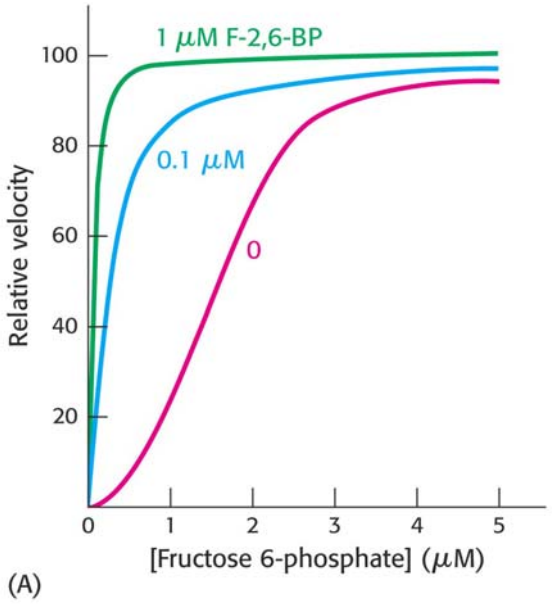



## PFK activity plotted as a function of [ATP]





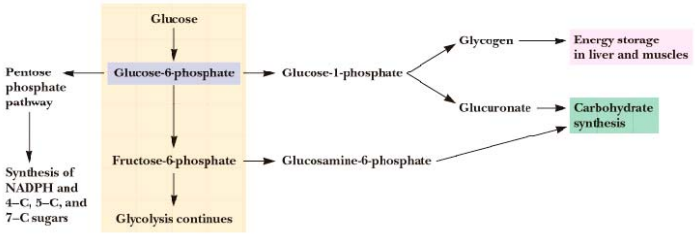

## Frc-2.6-BP effect on PFK activity



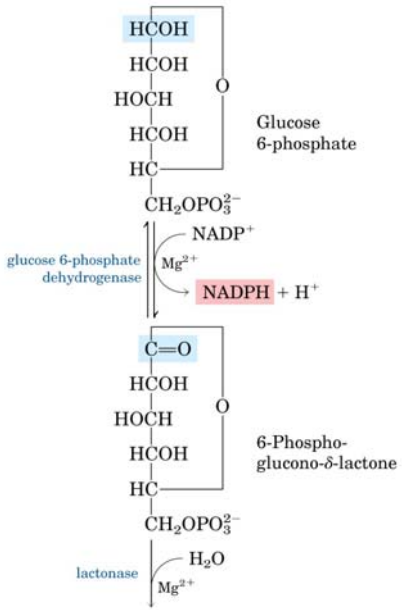


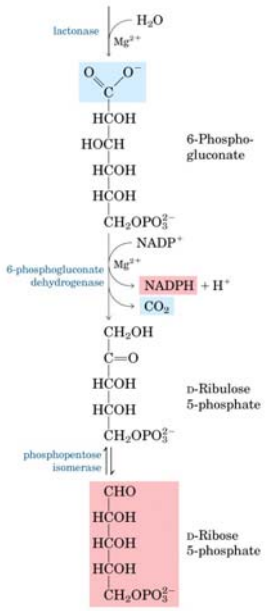

## Secondary pathways of glucose oxidation

- Primary: glycolysis
- Secondary: pentose phosphate pathway

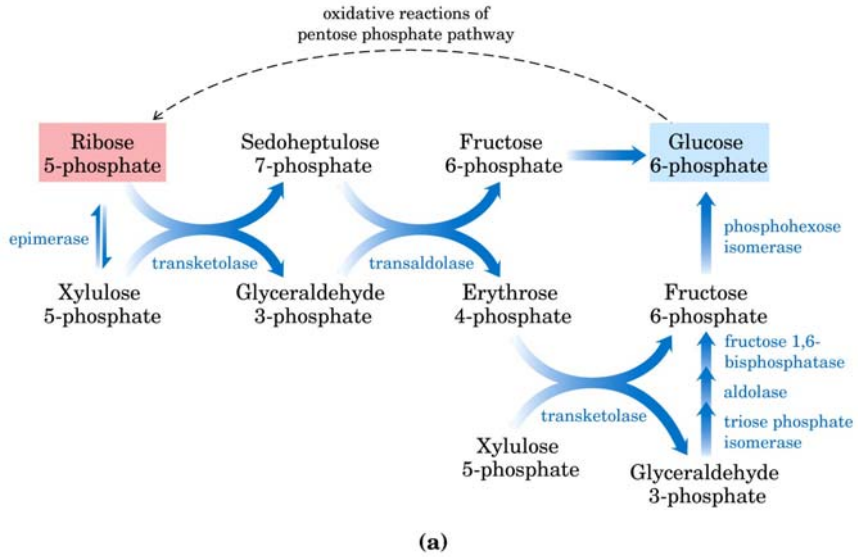




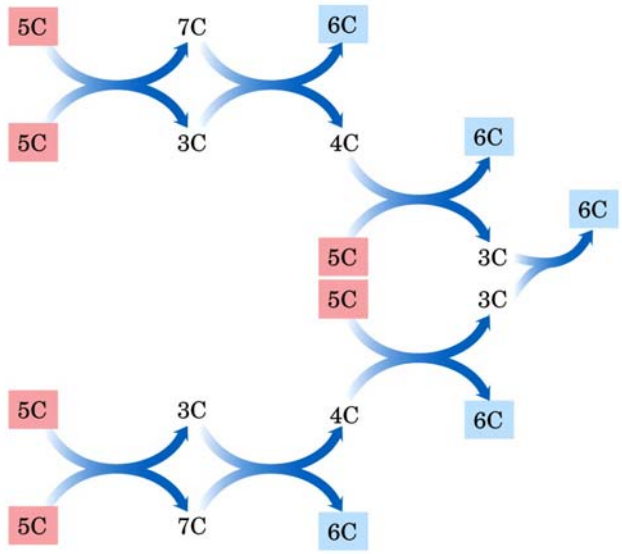

lactonase
$H_2O$
$Mg^{2+}$
HCOH
HOCH
HCOH
HCOH
$CH_2OPO_3^{2-}$
6-Phospho-gluconate
$NADP^+$
$Mg^{2+}$
6-phosphogluconate dehydrogenase
NADPH + $H^+$
$CO_2$
$CH_2OH$
C=O
HCOH
HCOH
$CH_2OPO_3^{2-}$
D-Ribulose 5-phosphate
phosphopentose isomerase
CHO
HCOH
HCOH
HCOH
$CH_2OPO_3^{2-}$
D-Ribose 5-phosphate




oxidative reactions of pentose phosphate pathway
Ribose 5-phosphate
Sedoheptulose 7-phosphate
Fructose 6-phosphate
Glucose 6-phosphate
epimerase
transketolase
transaldolase
phosphohexose isomerase
Xylulose 5-phosphate
Glyceraldehyde 3-phosphate
Erythrose 4-phosphate
Fructose 6-phosphate
fructose 1,6-bisphosphatase
aldolase
triose phosphate isomerase
transketolase
Xylulose 5-phosphate
Glyceraldehyde 3-phosphate

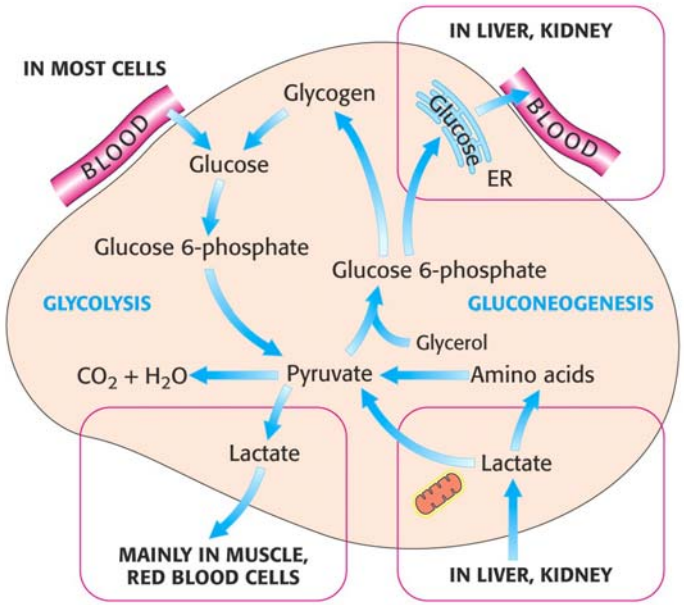
(a)




5C
7C
6C
5C
3C
4C
6C
6C
5C
3C
5C
3C
5C
3C
4C
6C
5C
7C
6C

(b)




IN LIVER, KIDNEY
IN MOST CELLS
BLOOD
Glycogen
Glucose
ER
BLOOD
Glucose 6-phosphate
Glucose 6-phosphate
GLYCOLYSIS
GLUCONEOGENESIS
Glycerol
$CO_2 + H_2O$
Pyruvate
Amino acids
Lactate
Lactate
MAINLY IN MUSCLE, RED BLOOD CELLS
IN LIVER, KIDNEY