Summer term 2016
Nanobiophysics Module

Introduction to Molecular and Cellular Biology

LECTURE 3-4:

Introduction to cell chemistry and biosynthesis II

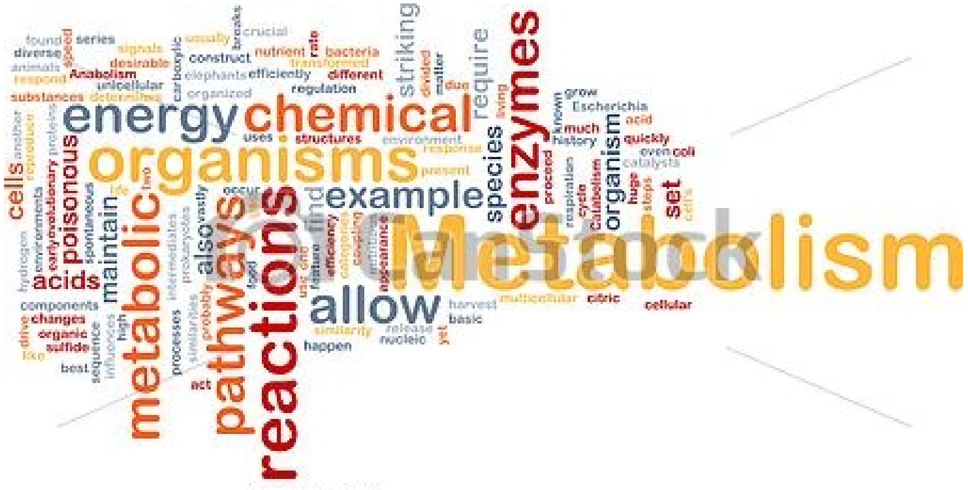
LECTURE 3-4: INTRODUCTION TO CELL CHEMISTRY AND BIOSYNTHESIS II

- **Principles of bioenergetics:**
  - **metabolism, anabolism, catabolism**
  - **metabolic reaction types**
  - **thermodynamics**
- **ATP hydrolysis**
- **Carriers in molecular biosythesis: ATP, NAD(P)H, FAD**
- **Oxidation-reduction reactions**
- **Examples of metabolic pathways:**
  - **photosynthesis**
  - **glycolysis**
  - **fermentation**

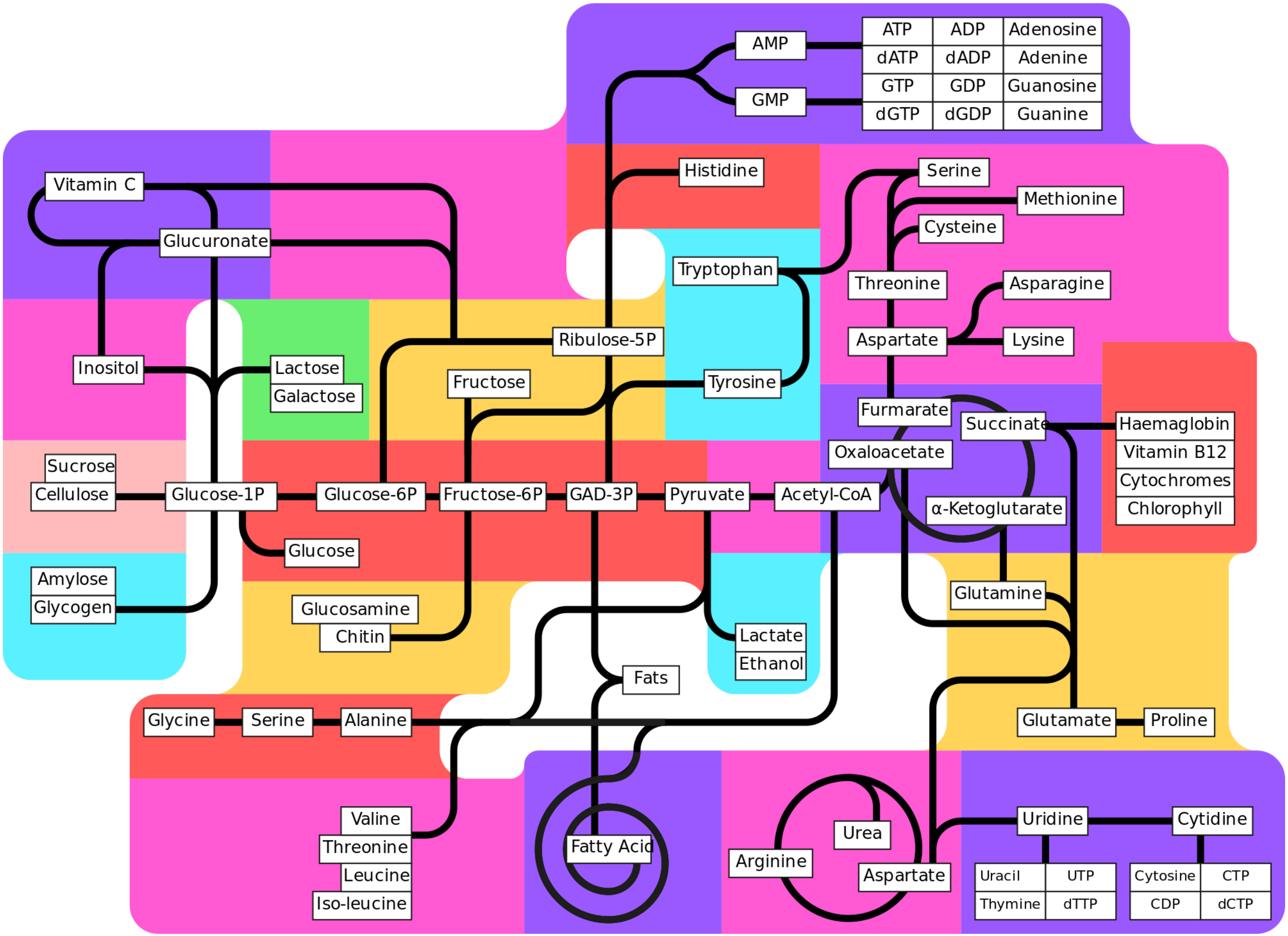
METABOLISM

Metabolism is a highly coordinated cell activity which includes many multienzyme systems cooperating to:

- **obtain solar energy or by degrading energy-rich nutrients**
- **convert nutrient molecules into macromolecules**
- **polymerize monomeric precursors into macromolecules**
- **synthesize/degrade biomolecules with specialized cellular function**



METABOLISM: GENERAL SCHEME



AMP

GMP

ATP	ADP	Adenosine
dATP	dADP	Adenine
GTP	GDP	Guanosine
dGTP	dGDP	Guanine

Vitamin C

Glucuronate

Histidine

Serine

Methionine

Cysteine

Tryptophan

Threonine

Asparagine

Aspartate

Lysine

Ribulose-5P

Inositol

Lactose

Galactose

Fructose

Tyrosine

Furmarate

Succinate

Oxaloacetate

α-Ketoglutarate

Haemaglobin

Vitamin B12

Cytochromes

Chlorophyll

Sucrose

Cellulose

Glucose-1P

Glucose-6P

Fructose-6P

GAD-3P

Pyruvate

Acetyl-CoA

Glucose

Amylose

Glycogen

Glucosamine

Chitin

Glutamine

Lactate

Ethanol

Fats

Glycine

Serine

Alanine

Glutamate

Proline

Valine

Threonine

Leucine

Iso-leucine

Fatty Acid

Urea

Arginine

Aspartate

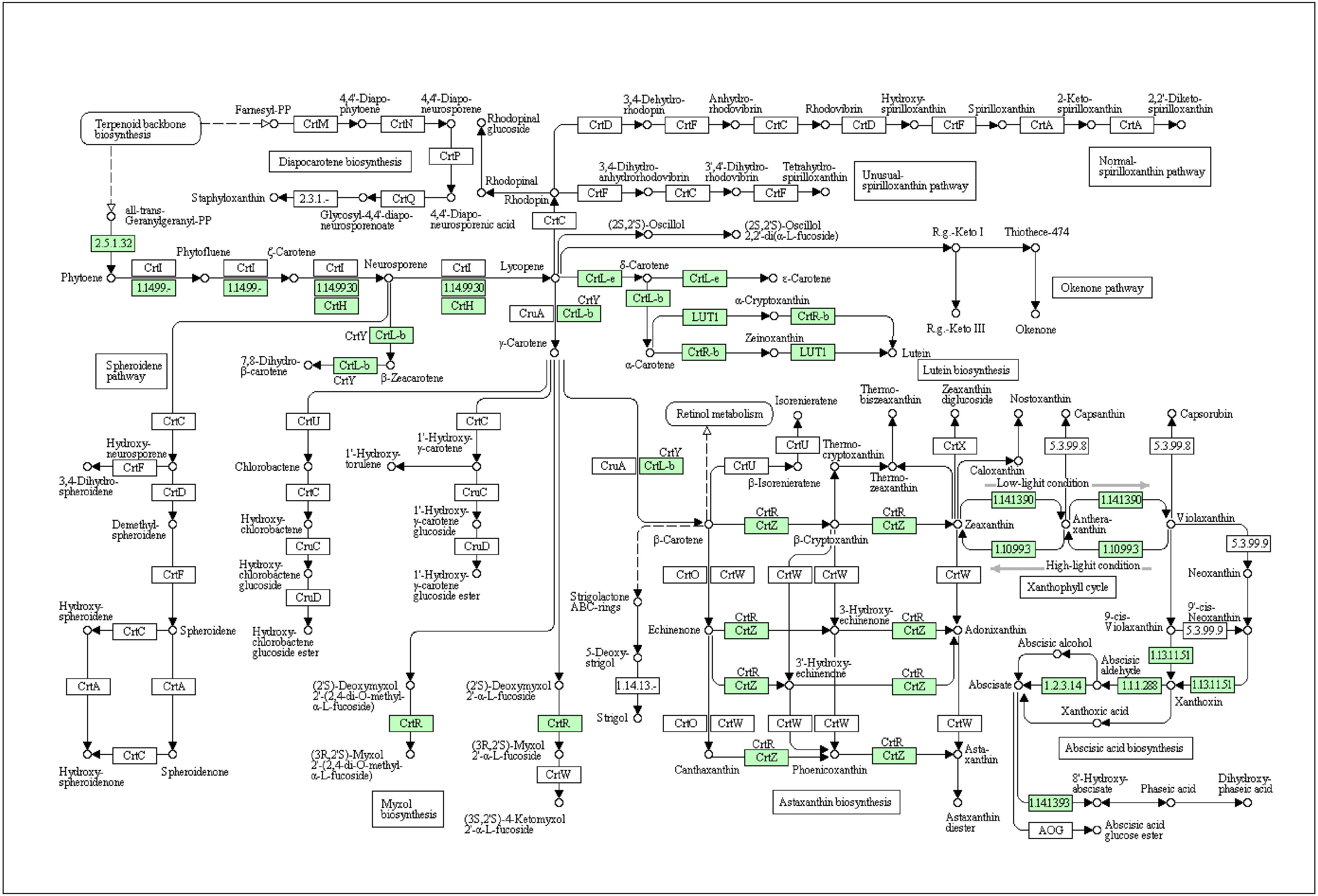
Uridine

Cytidine

Uracil	UTP
Thymine	dTTP

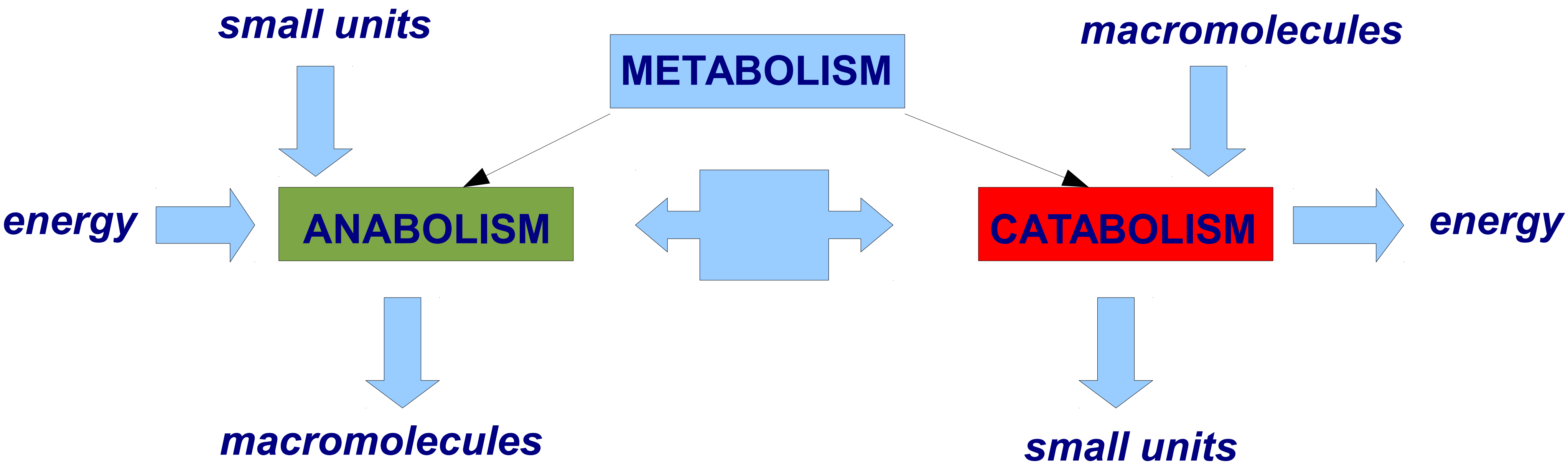
Cytosine	CTP
CDP	dCTP

METABOLISM EXAMPLE: CAROTENOID



ANABOLISM/CATABOLISM

- **Anabolism: metabolic pathways constructing macmolecules from small units**
- **Catabolism: metabolic pathways breaking down macromolecules to small units to release energy or to be used in anabolic reactions.**


small units

METABOLISM

macromolecules

energy

ANABOLISM

CATABOLISM

energy

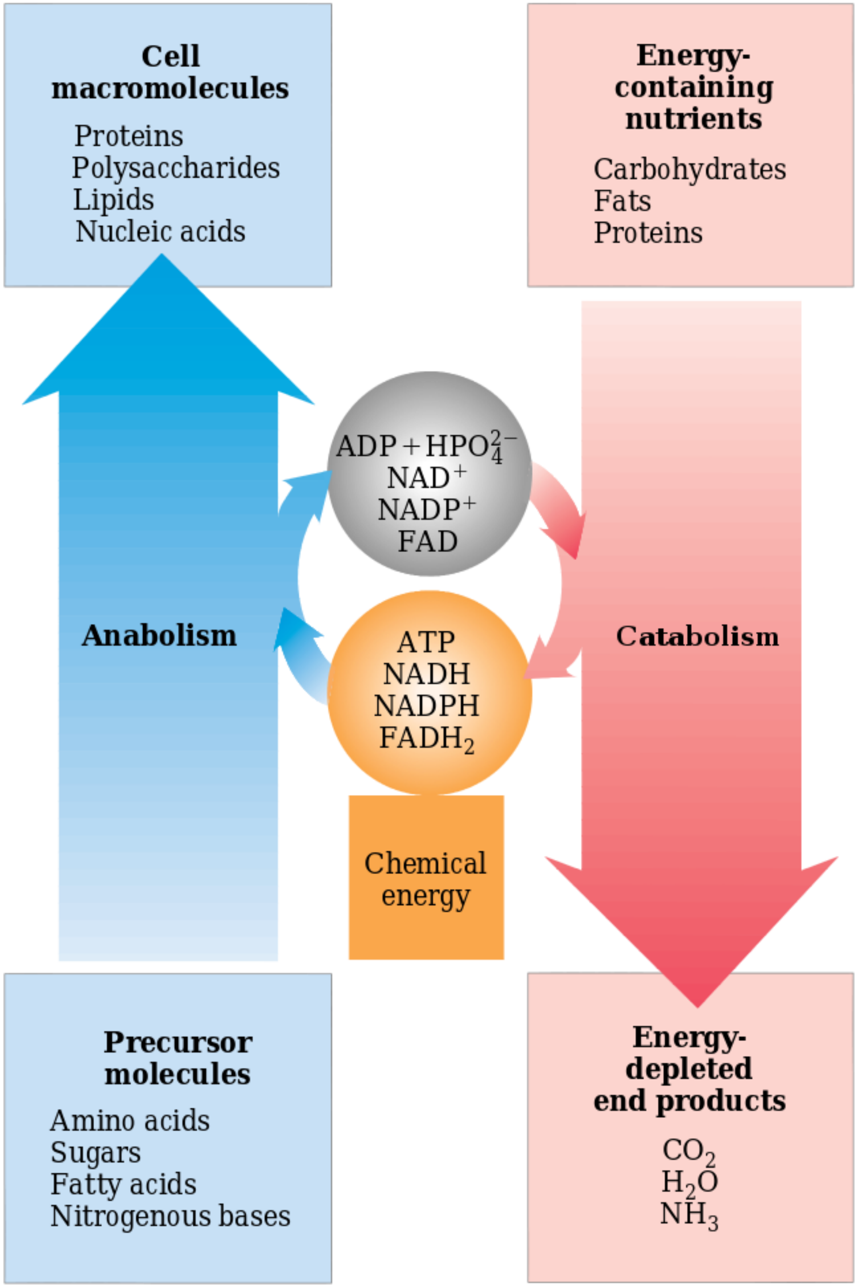
macromolecules

small units

Solving problem of reversibility of catabolic/anabolic reactions:

- **Separate regulation**
- **High energy barrier of one/several of steps**
- **Different localization**

ANABOLISM/CATABOLISM



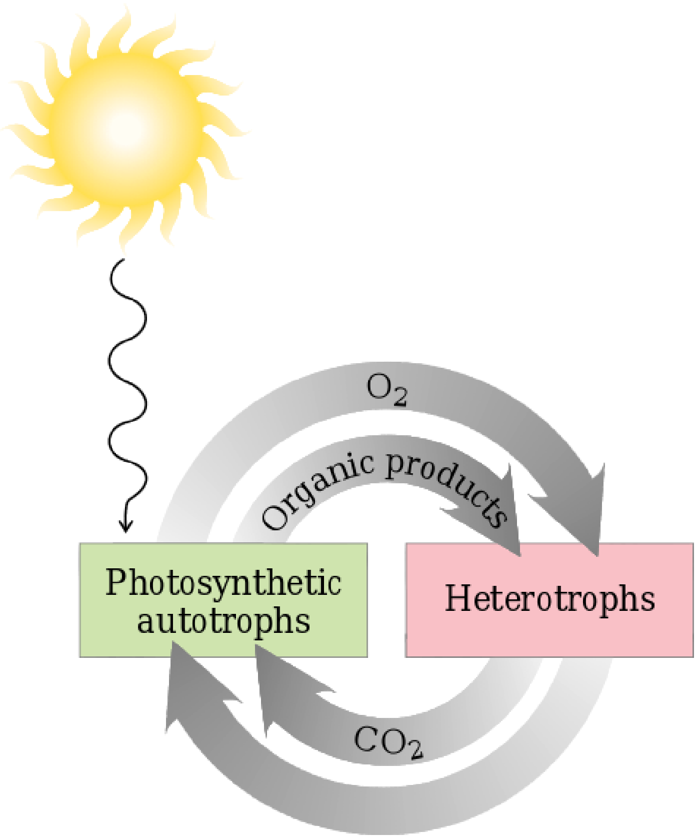
CARBON SOURCES

- **Autotrophs: C from atmosphere + sun energy => macromolecules**

  - photosynthetic bacteria and plants

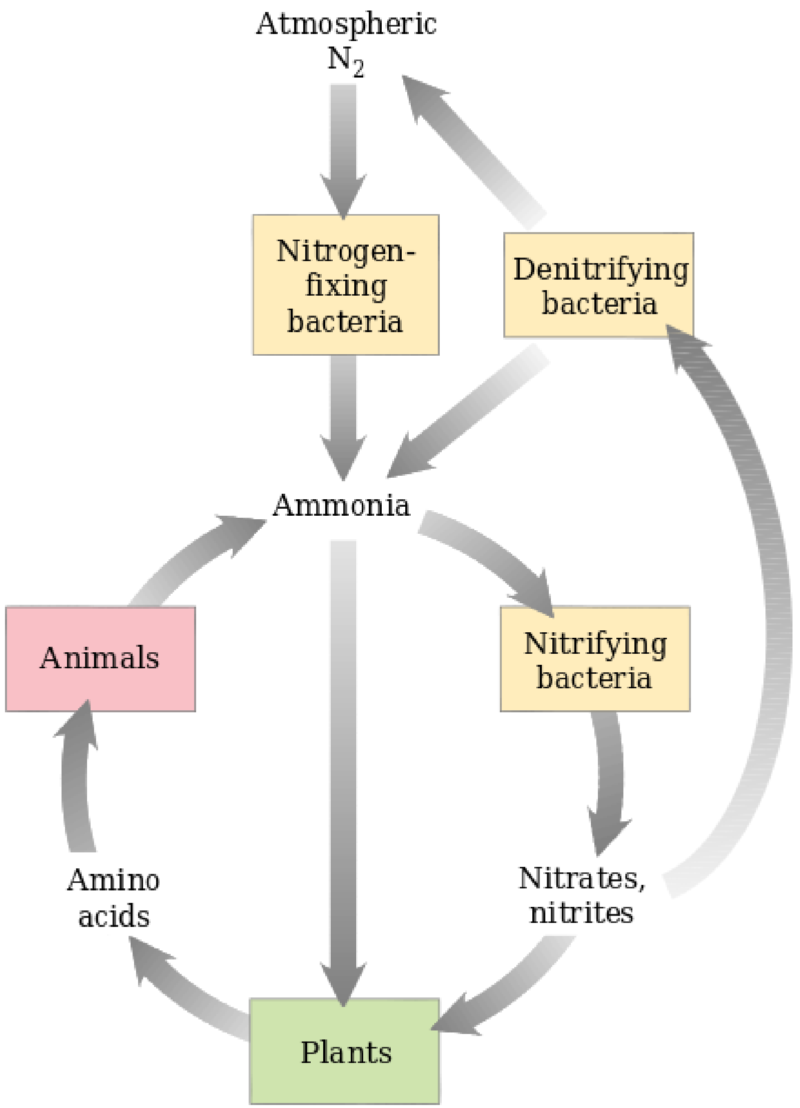
- **Heterotrophs: cannot do this independently**

  - animals and most microorganisms



NITROGEN CYCLING

Very few microorganisms are able to “fix” N from the athmosphere.

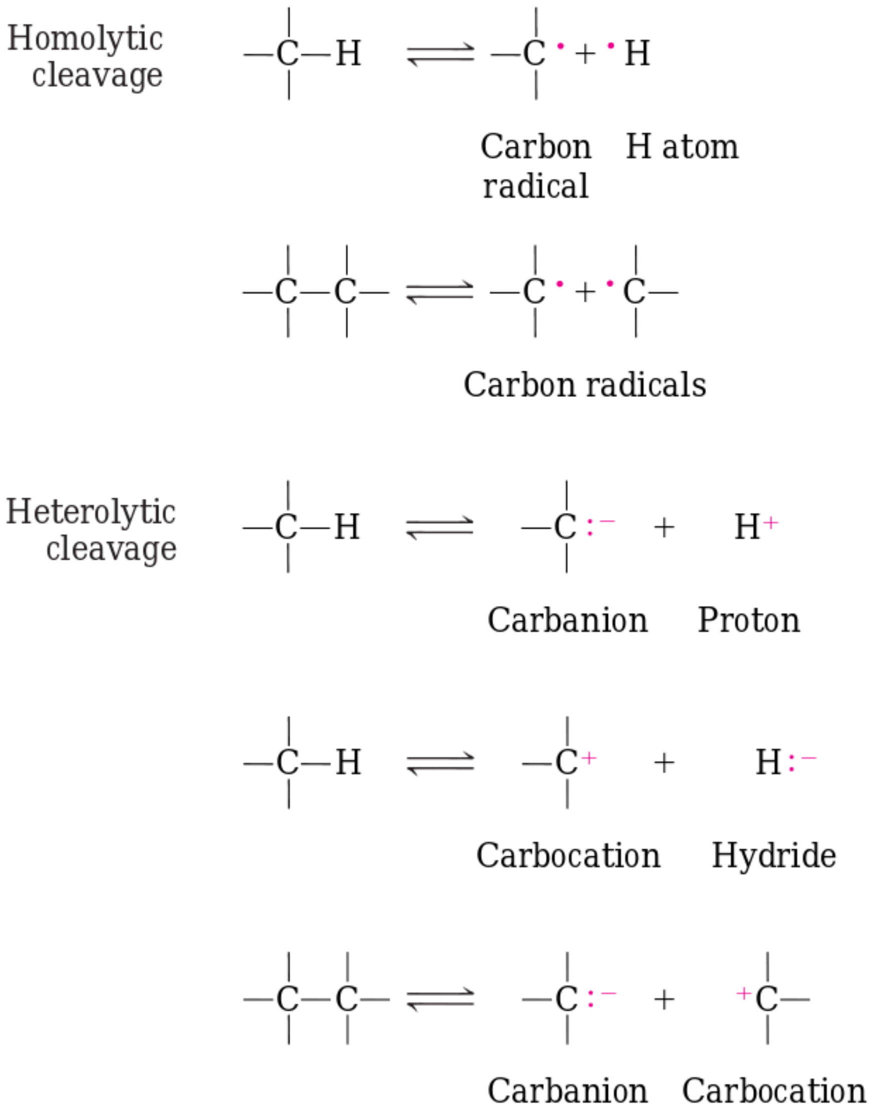


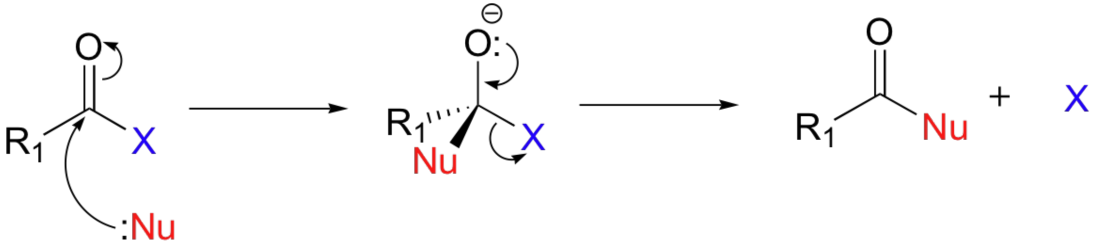
REGULATION OF METABOLIC REACTIONS

S (substrate) <=> P (product)

- Substrate concentration level
- Enzyme concentration level
- Compartmentalization of the reaction
- Energy supply
- Growth factors/hormone
- Allosteric regulation (f.i. phosphorylation)

CLASSES OF CHEMICAL REACTIONS: MAIN MECHANISMS

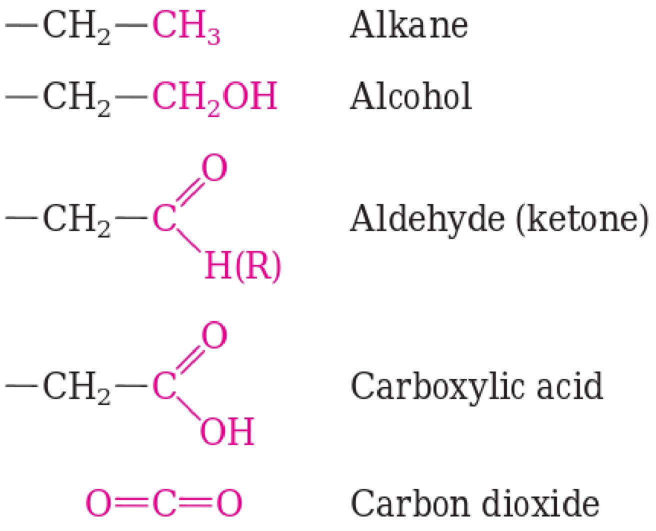


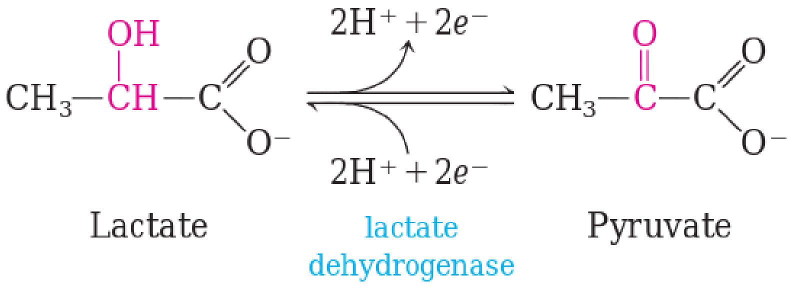


Nucleophilic reaction

CLASSES OF CHEMICAL REACTIONS

- **Oxidation/reduction**
- **Formation/breaking C-C bonds**
- **Internal rearrangements, isomerizations, eliminations**
- **Group transfer reactions**
- **Free radical reactions**





- electron acceptors/donors

- energy consume/production

CLASSES OF CHEMICAL REACTIONS

- Oxidation/reduction
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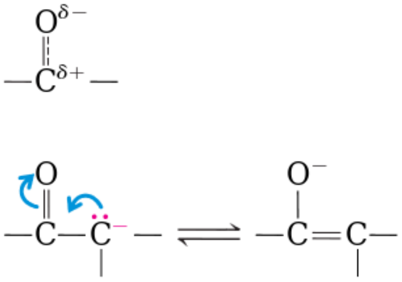
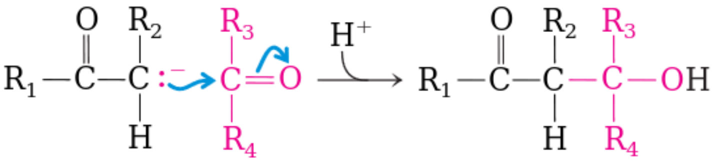
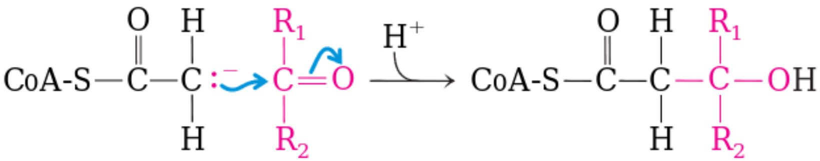
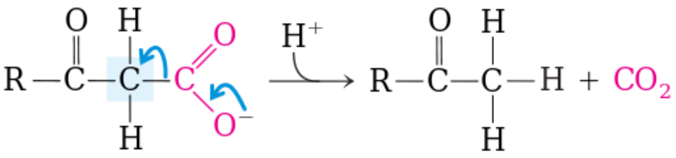




Aldol condensation



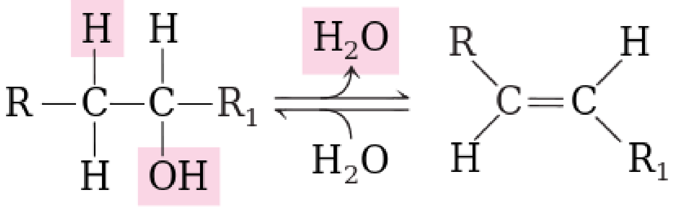
Claisen ester condensation



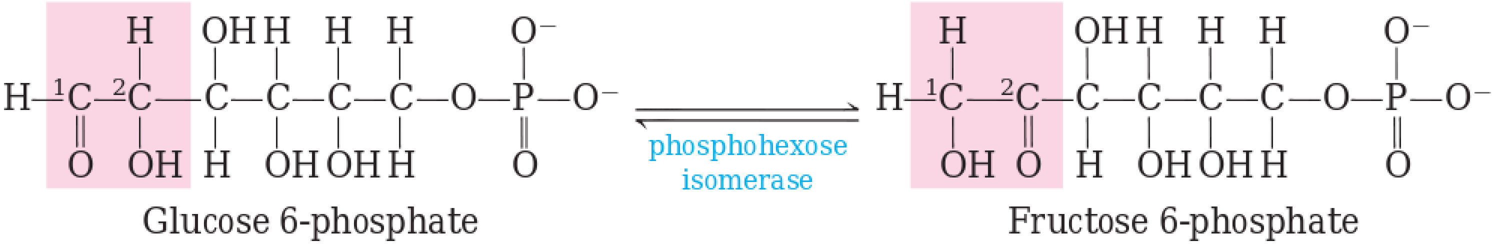
Decarboxylation of a β-keto acid

CLASSES OF CHEMICAL REACTIONS

- **Oxidation/reduction**
- **Formation/breaking C-C bonds**
- **Internal rearrangements, isomerizations, eliminations**
- **Group transfer reactions**
- **Free radical reactions**



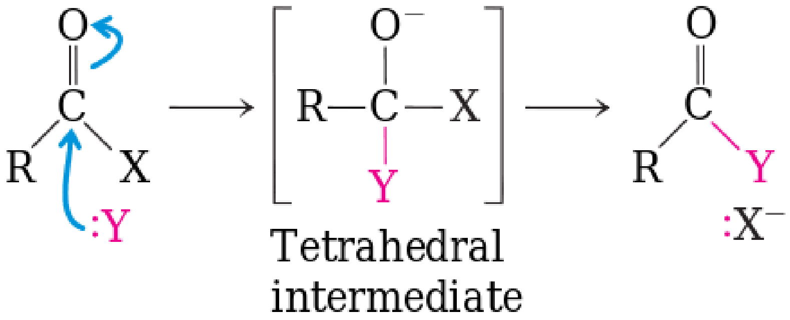
Elimination



Isomerization

CLASSES OF CHEMICAL REACTIONS

- **Oxidation/reduction**
- **Formation/breaking C-C bonds**
- **Internal rearrangements, isomerizations, eliminations**
- **Group transfer reactions**
- **Free radical reactions**



CLASSES OF CHEMICAL REACTIONS

- Oxidation/reduction
- Formation/breaking C-C bonds
- Internal rearrangements, isomerizations, eliminations
- Group transfer reactions
- Free radical reactions

$$A\cdot + \cdot B \Leftrightarrow A{:}B$$

THERMODYNAMICS

- **First Law: for any physical or chemical change, the total amount of energy in the universe remains constant; energy may change form or it may be transported from one region to another, but it cannot be created or destroyed.**
- **Second Law: in all natural processes, the entropy of the universe increases.**

$$\Delta G = \Delta H - T\Delta S$$

ORDER
(low S)

THERMODYNAMICS

- ΔG- Gibbst free energy (T=const, p=const):

  - exergonic reactions, $\Delta G<0$

  - endergonic reactions, $\Delta G>0$

- H- enthalpy (heat content of the reaction)

  - exothermic reactions, $\Delta H<0$

  - endothermic reactions, $\Delta H>0$

- S- entropy (order measure of the system)

THERMODYNAMICS

aA + bB <=> cC + dD

K_{eq}= $[C]^c[D]^d/[A]^a[B]^b$

$\Delta G = -RT \ln K_{eq}$

$\Delta G'^0$ standard free energy change (pH 7, T=298 K, $[H_2O]$=55.5 M)

$\Delta G'^0 = -RT \ln K'_{eq}$

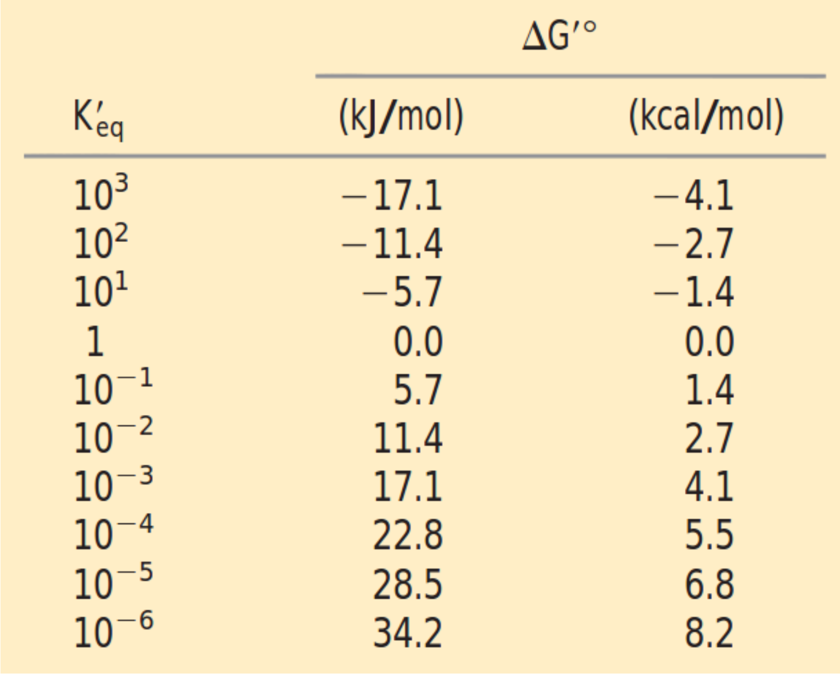
Actual free energy change:

A + B <=> C + D

$\Delta G = \Delta G'^0 + RT \ln ([C][D]/[A][B])$

In equilibrium:

$0 = \Delta G = \Delta G'^0 + RT \ln ([C]_{eq}[D]_{eq}/[A]_{eq}[B]_{eq})$

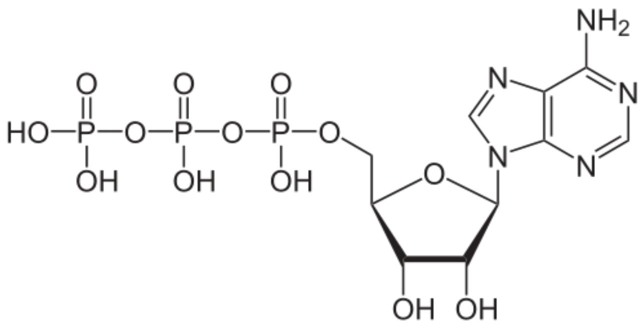


	$\Delta G'^\circ$	
K'_{eq}	(kJ/mol)	(kcal/mol)
10^3	−17.1	−4.1
10^2	−11.4	−2.7
10^1	−5.7	−1.4
1	0.0	0.0
10^{-1}	5.7	1.4
10^{-2}	11.4	2.7
10^{-3}	17.1	4.1
10^{-4}	22.8	5.5
10^{-5}	28.5	6.8
10^{-6}	34.2	8.2

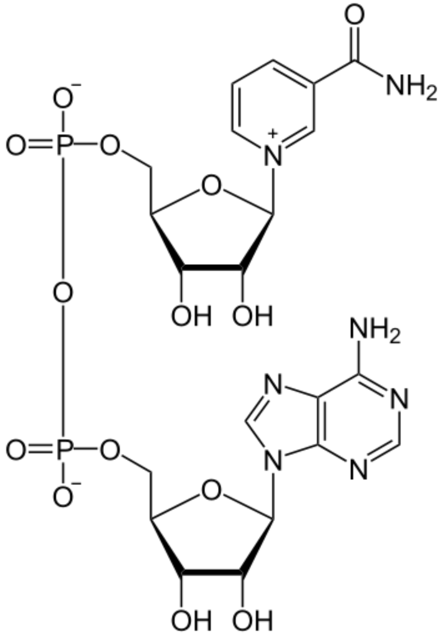
1 cal = 4.184 J

CARRIERS IN MOLECULAR BIOSYNTHESIS

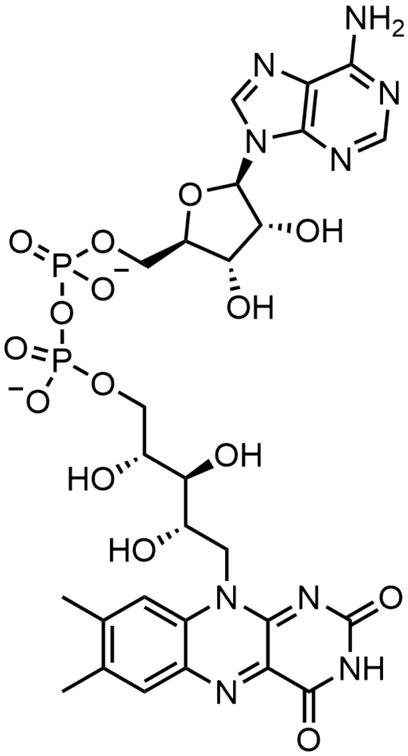
- **ATP (Adenosine triphosphate)**



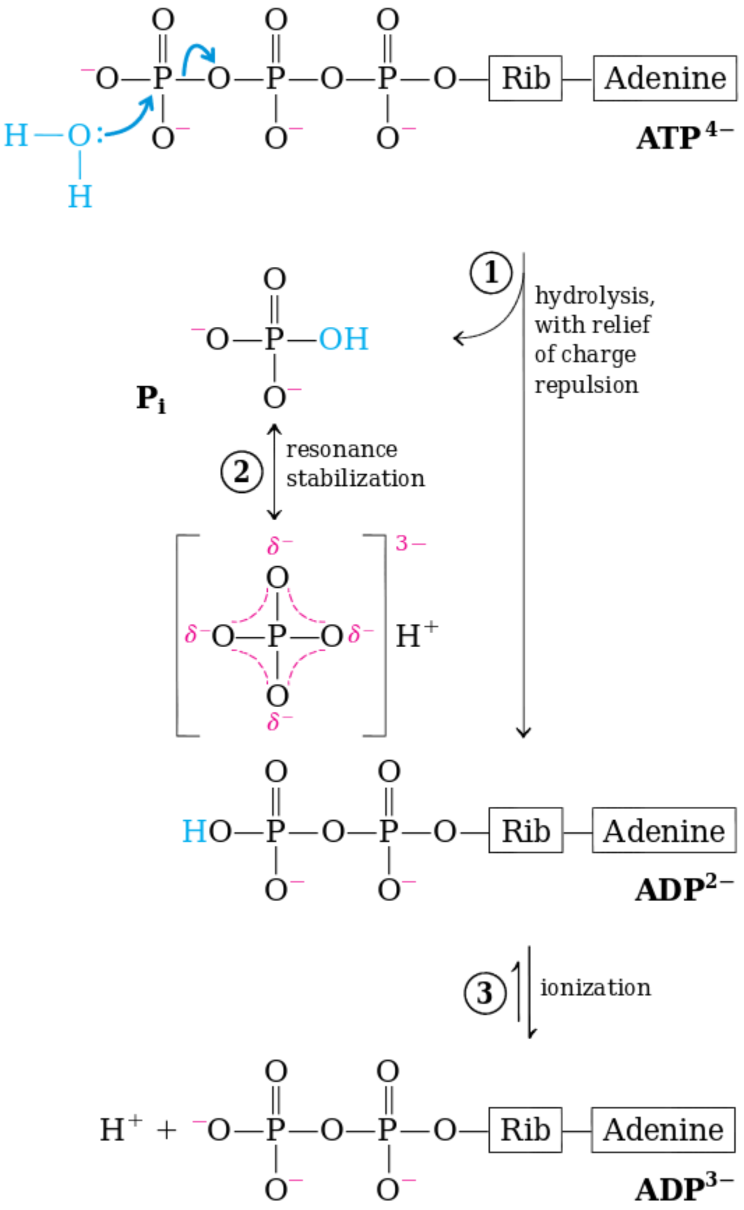
- **NADH/NADHP (Nicotinamide adenine dinucleotide)**



- **FAD (Flavin adenine dinucleotide)**

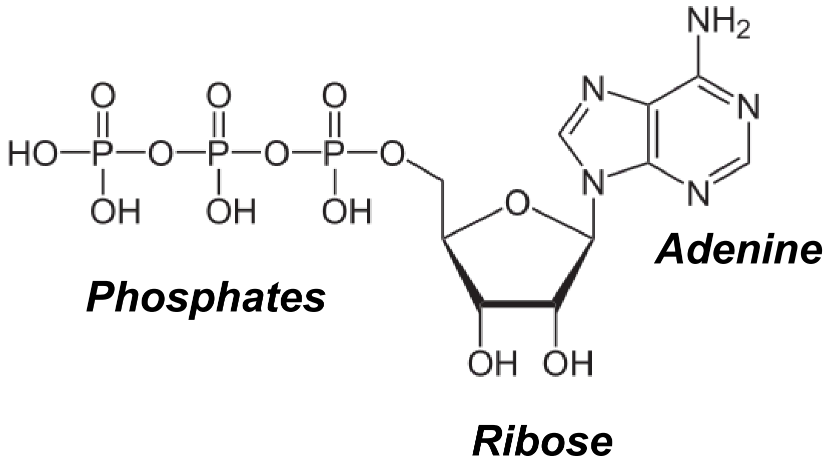


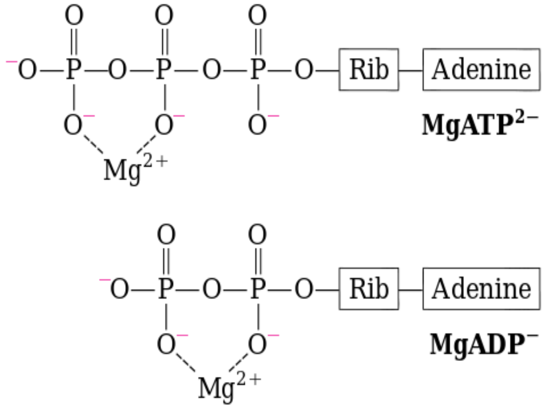
ATP: BASIS OF CHEMICAL HYDROLYSIS



$$ATP^{4-} + H_2O \longrightarrow ADP^{3-} + P_i^{2-} + H^+$$

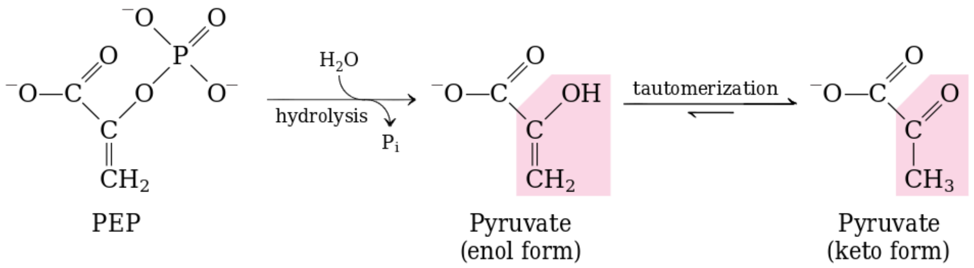
$$\Delta G'^\circ = -30.5 \text{ kJ/mol}$$





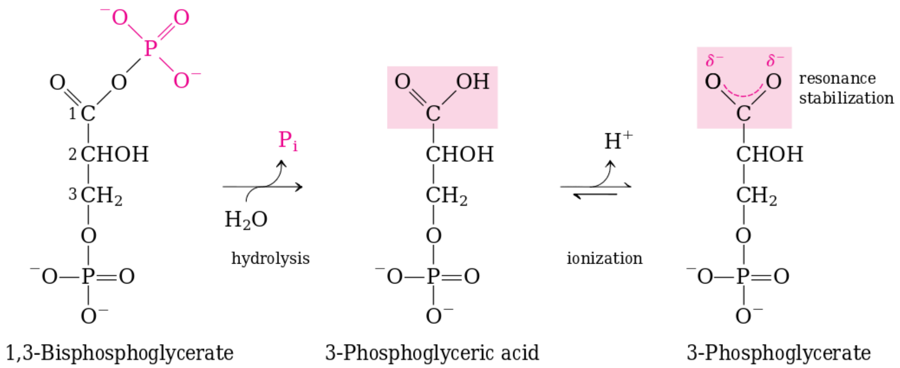
- High activation barrier
- Concentrations are important
- Mg^{2+} impact
- ΔG_p = [-50 ; 65 kJ/mol] in intact cells

OTHER HYDROLYZED COMPOUNDS



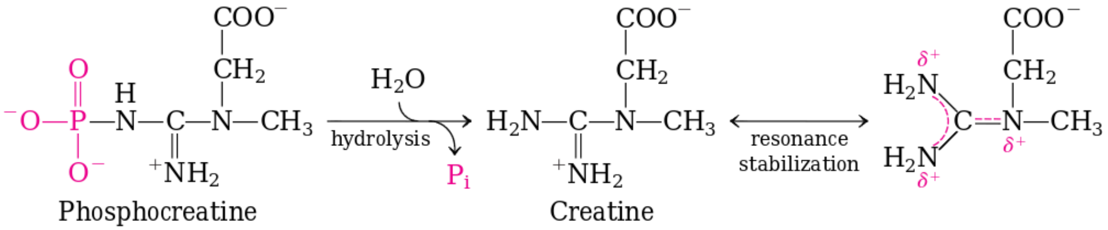
$$PEP^{3-} + H_2O \longrightarrow \text{pyruvate}^{-} + P_i^{2-}$$
$$\Delta G'^{\circ} = -61.9\ \text{kJ/mol}$$

Phosphoenolpyruvate



$$\text{1,3-Bisphosphoglycerate}^{4-} + H_2O \longrightarrow \text{3-phosphoglycerate}^{3-} + P_i^{2-} + H^{+}$$
$$\Delta G'^{\circ} = -49.3\ \text{kJ/mol}$$

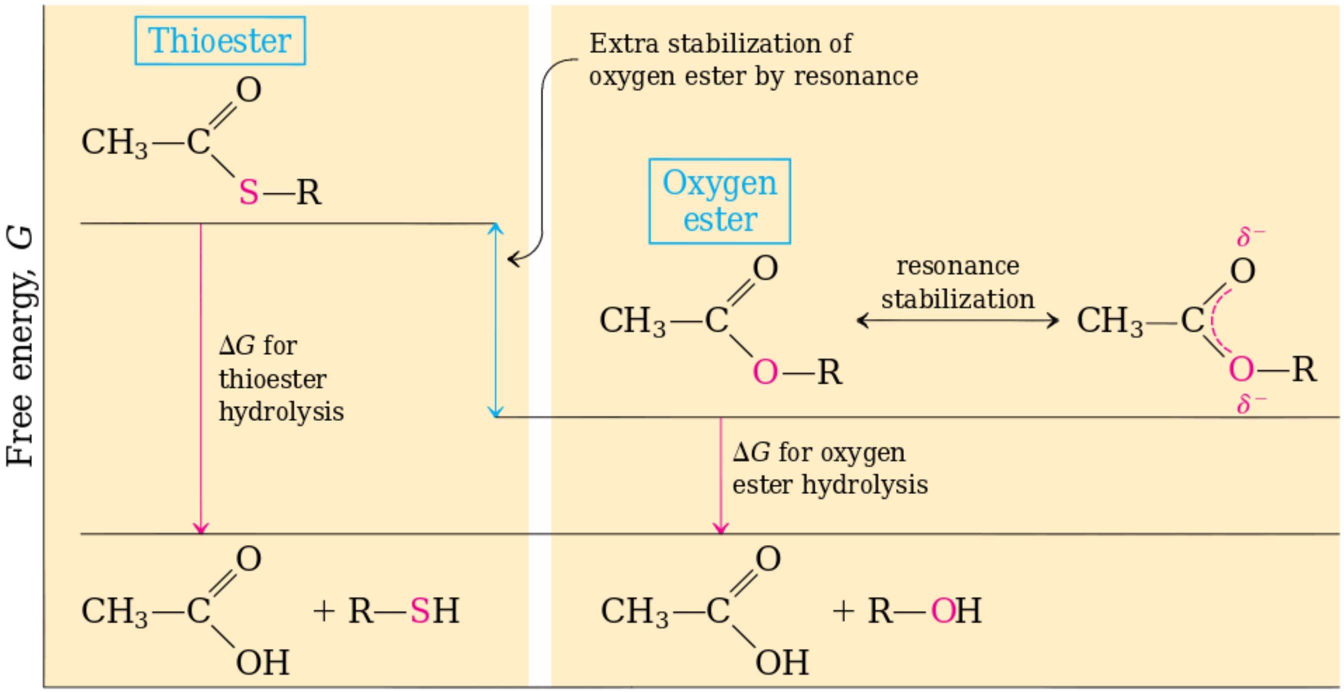
1,3-Bisphosphoglycerate

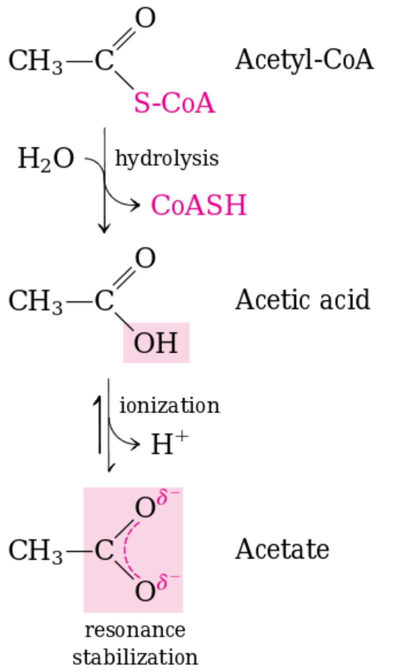


$$\text{Phosphocreatine}^{2-} + H_2O \longrightarrow \text{creatine} + P_i^{2-}$$
$$\Delta G'^{\circ} = -43.0\ \text{kJ/mol}$$

Phosphocreatine

THIOESTERS AND ESTERS





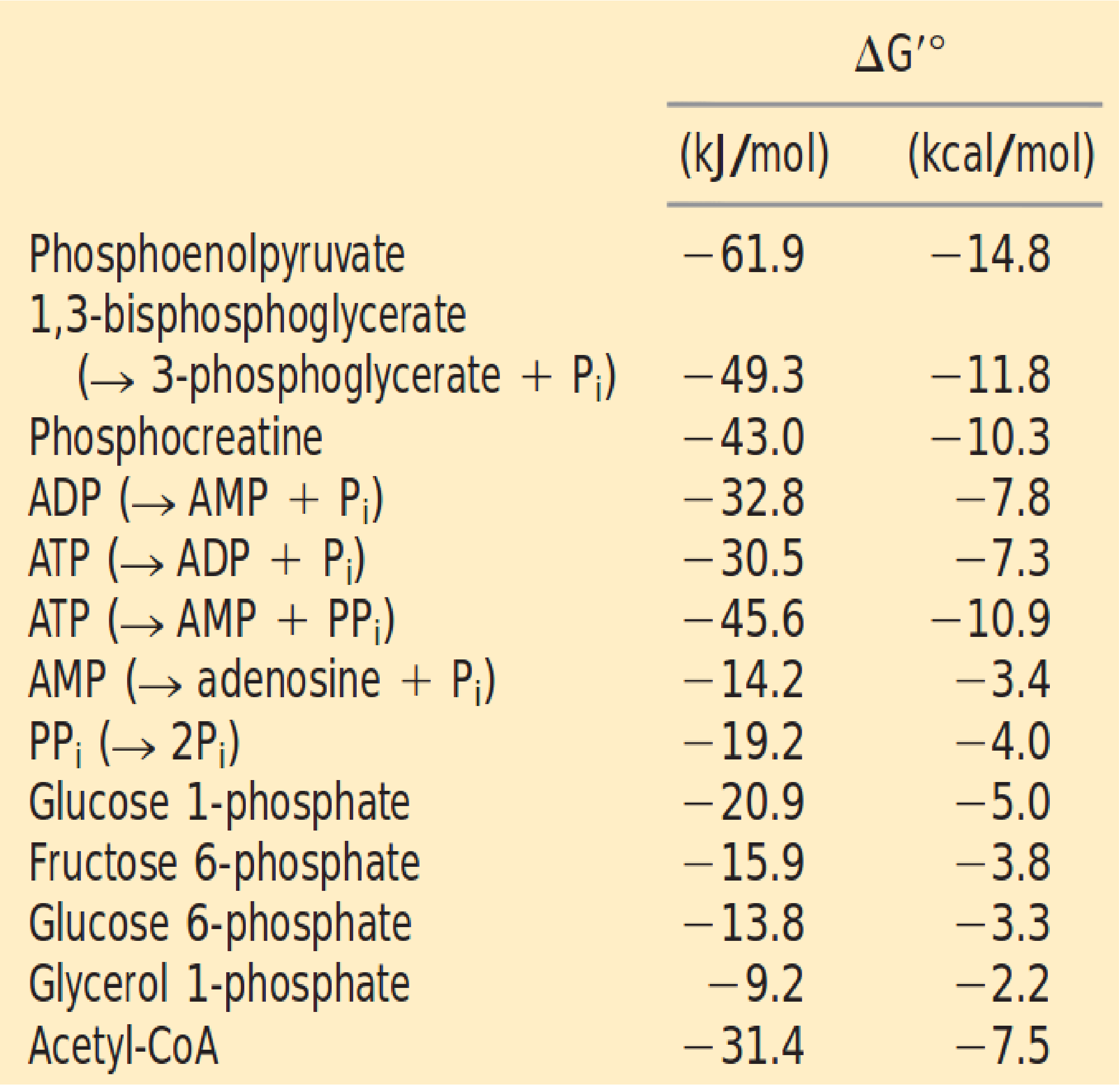
$$\text{Acetyl-CoA} + H_2O \longrightarrow \text{acetate}^- + \text{CoA} + H^+$$
$$\Delta G'^\circ = -31.4\ \text{kJ/mol}$$

Acetyl-CoA

HYDROLYSIS REACTIONS: SUMMARY

- The bond strain in reactants due to electrostatic repulsion is relieved by charge separation, as for ATP.
- The products are stabilized by ionization, as for ATP, acyl phosphates, and thioesters.
- The products are stabilized by isomerization (tautomerization), as for phosphoenolpyruvate.
- The products are stabilized by resonance, as for creatine released from phosphocreatine, carboxylate ion released from acyl phosphates and thioesters, and P_i released from anhydride or ester linkages.

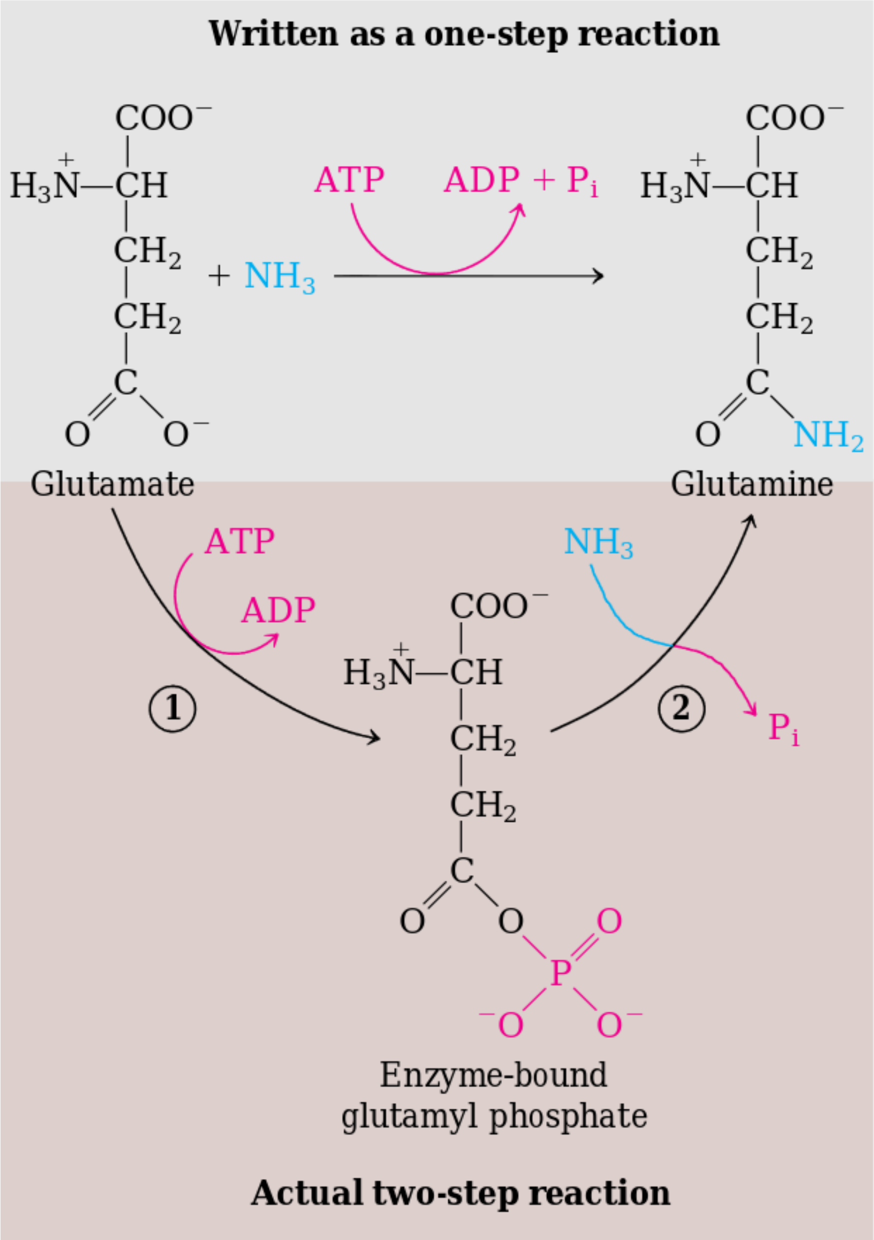
HYDROLYSIS REACTIONS: COMPARISON



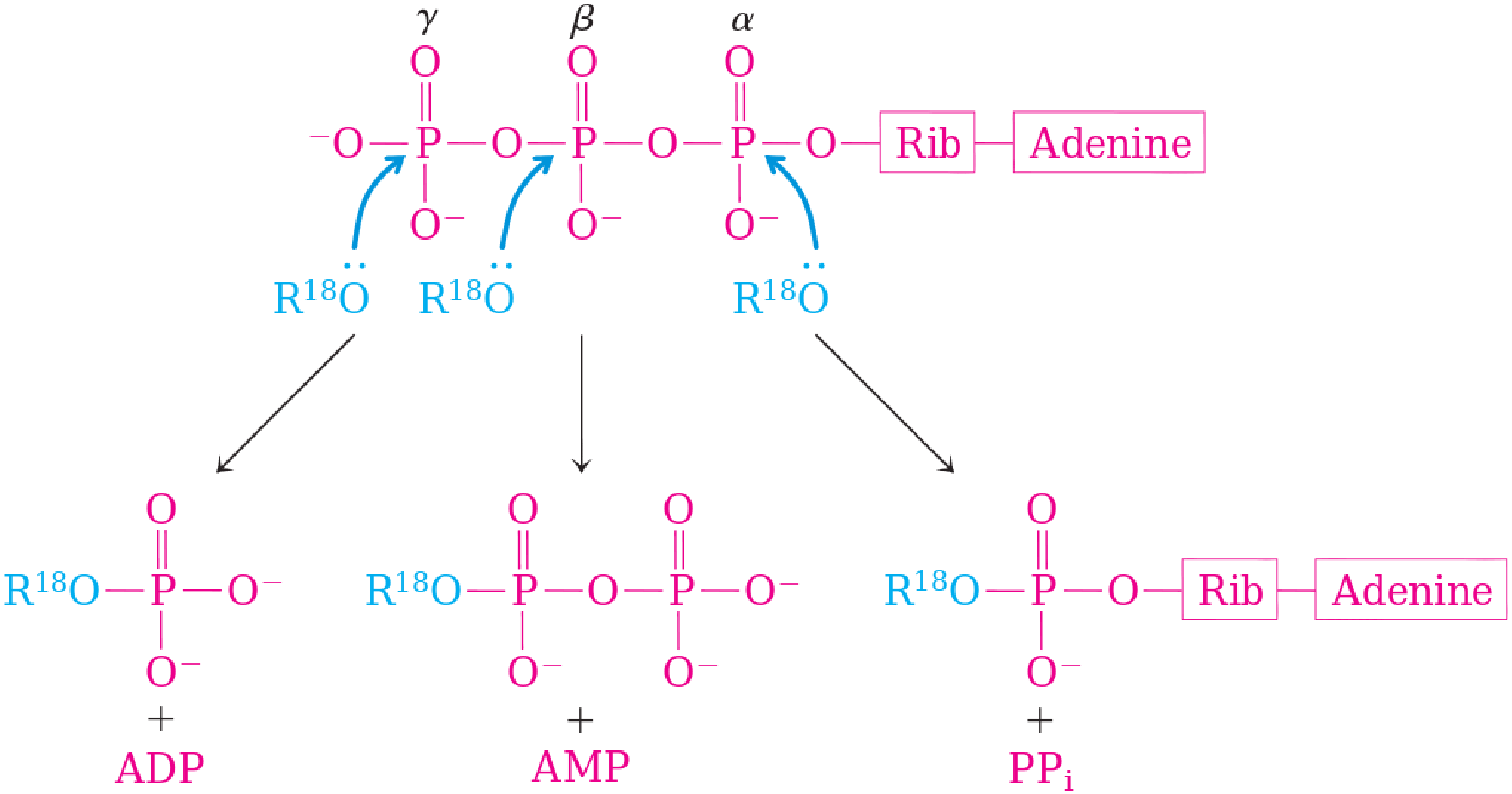
	$\Delta G'^\circ$	
	(kJ/mol)	(kcal/mol)
Phosphoenolpyruvate	−61.9	−14.8
1,3-bisphosphoglycerate (→ 3-phosphoglycerate + P_i)	−49.3	−11.8
Phosphocreatine	−43.0	−10.3
ADP (→ AMP + P_i)	−32.8	−7.8
ATP (→ ADP + P_i)	−30.5	−7.3
ATP (→ AMP + PP_i)	−45.6	−10.9
AMP (→ adenosine + P_i)	−14.2	−3.4
PP_i (→ $2P_i$)	−19.2	−4.0
Glucose 1-phosphate	−20.9	−5.0
Fructose 6-phosphate	−15.9	−3.8
Glucose 6-phosphate	−13.8	−3.3
Glycerol 1-phosphate	−9.2	−2.2
Acetyl-CoA	−31.4	−7.5

Important is to sum up all the reaction components to find out final free energy outcome!

ATP HYDROLYSIS INVOLVES GROUP TRANSFERS



ATP: SEVERAL WAYS OF GROUPS DONATION



ADP

Phosphoryl
transfer

AMP

Pyrophosphoryl
transfer

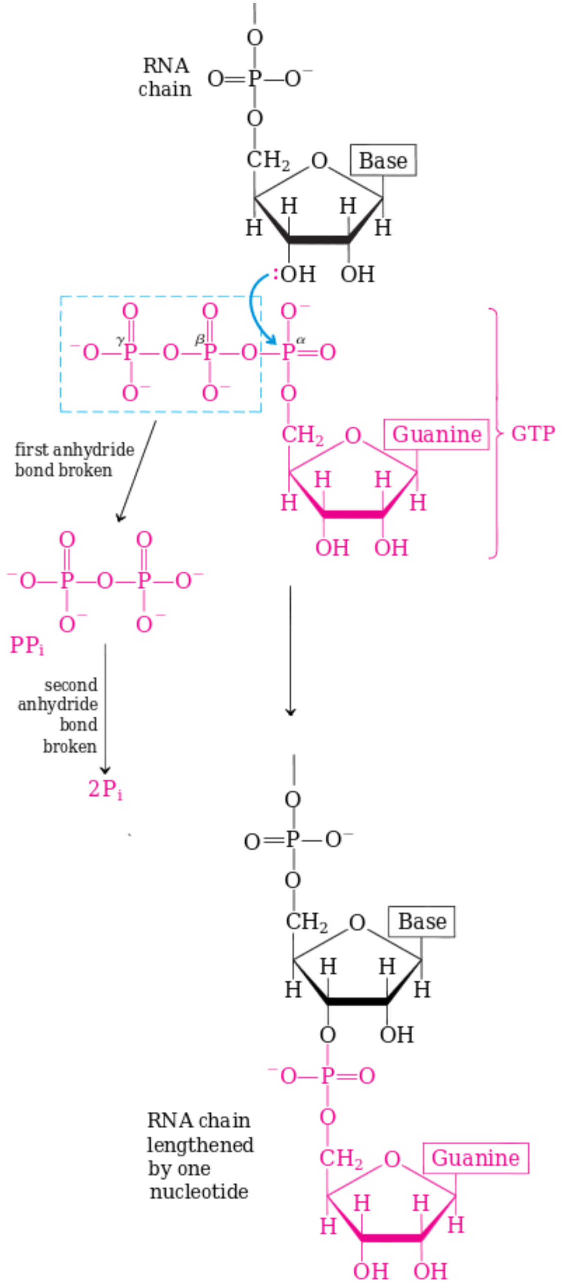
PP_i

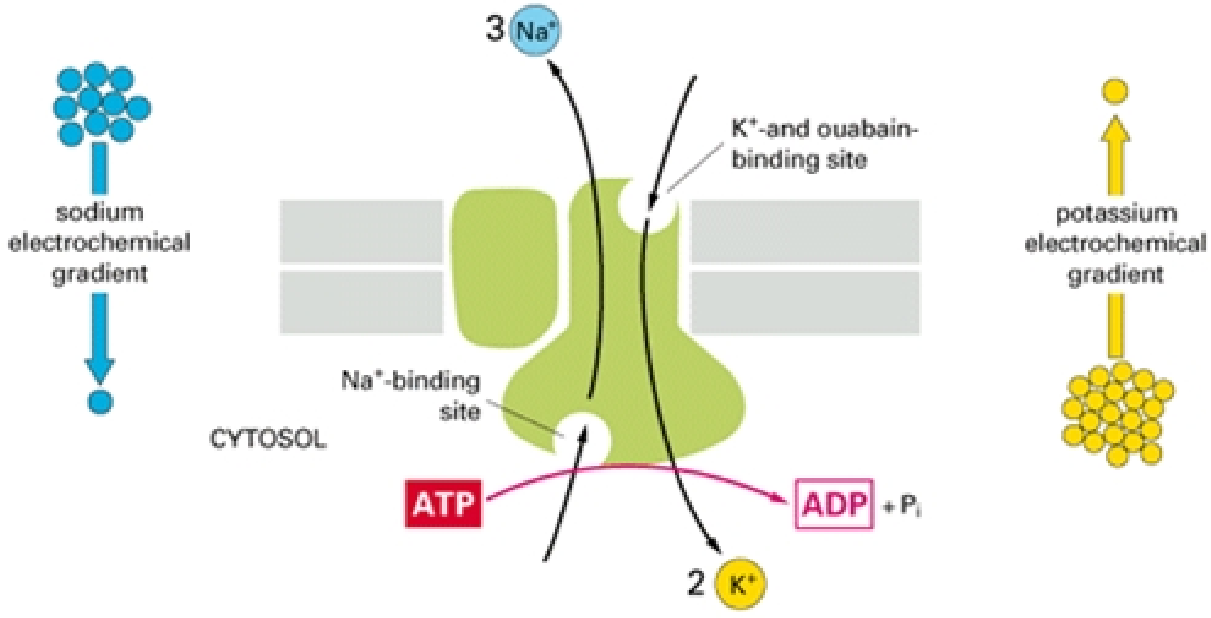
Adenylyl
transfer

Most favourable energetically

ATP HYDROLYSIS DEPENDENT PROCESSES

- **DNA/RNA synthesis**
- **AA activation (adenylation)**
- **Active ion transfer (Na^+/K^+ ATPase)**
- **Contraction of muscle fibers**





ATP: TRANSPHOSPHORYLATION

- **Nucleotide diphosphate kinase (+ Mg^{2+}):**

  ATP + NDP <=> ADP + NTP; $\Delta G'^0 \approx 0$

- **Adenylate kinase (+ Mg^{2+}):**

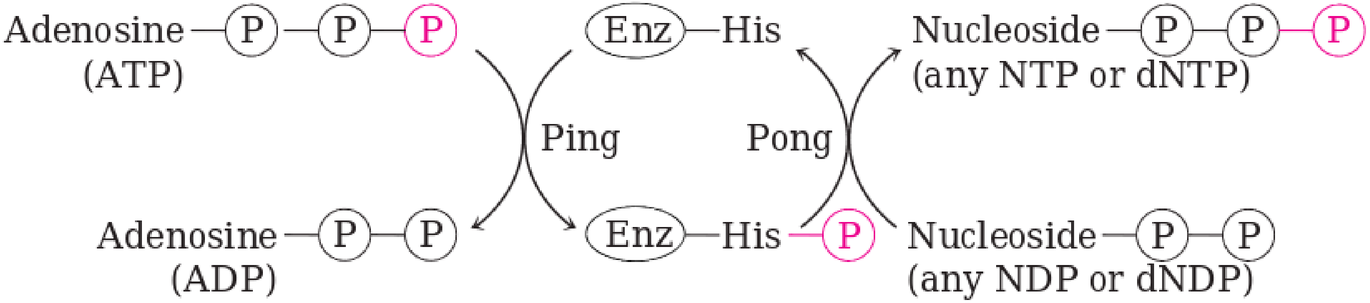
  2ADP <=> ATP + AMP; $\Delta G'^0 \approx 0$

- **Creatine kinase (+ Mg^{2+}):**

  ADP + P-Cr <=> ATP + Cr; $\Delta G'^0 = -12.5$ kJ/mol

- **Prokaryotic polyphosphate kinase 1/2 (+ Mg^{2+}):**

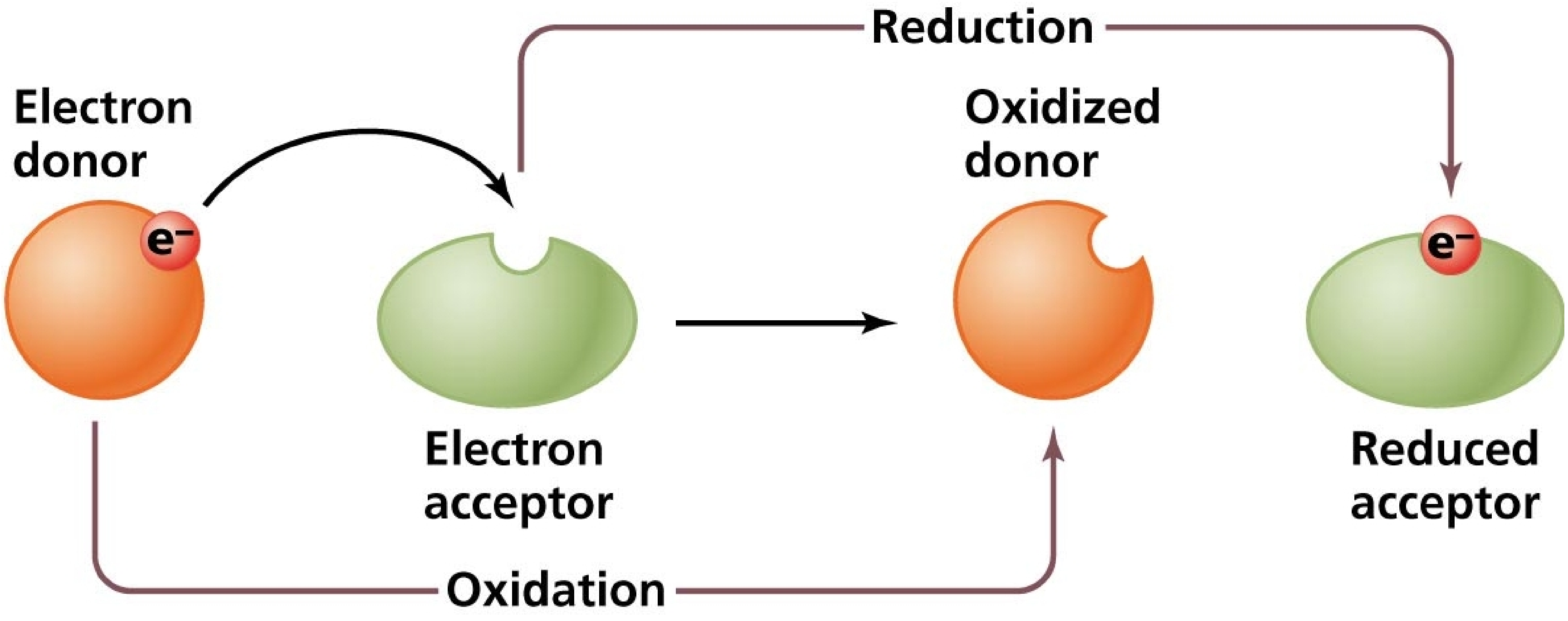
  ATP/GDP + $polyP_n/P_{n+1}$ <=> ADP/GTP + $polyP_{n+1}/P_n$



Nucleotide diphosphate kinase

OXIDATION-REDUCTION REACTIONS

- Lost of electrons: oxidation
- Gain of electrons: reduction



Electromotive force ~ difference in electron affinity

OXIDATION-REDUCTION REACTIONS AS HALF-REACTIONS: EXAMPLES

$Fe^{2+} + Cu^{2+}$ <=> $Fe^{3+} + Cu^{+}$

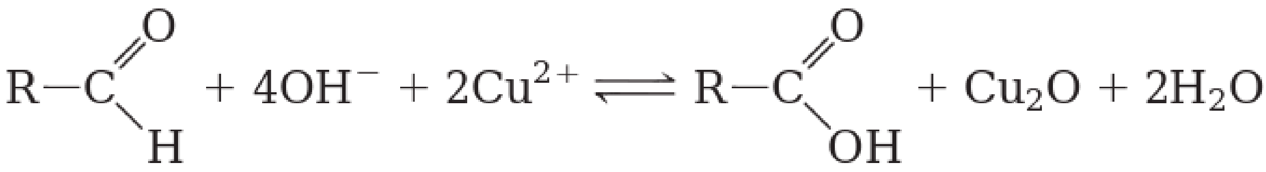
(1) Fe^{2+} <=> $Fe^{3+} + e^{-}$

(2) $Cu^{2+} + e^{-}$ <=> Cu^{+}

Fe^{2+} - reducing agent, reductant

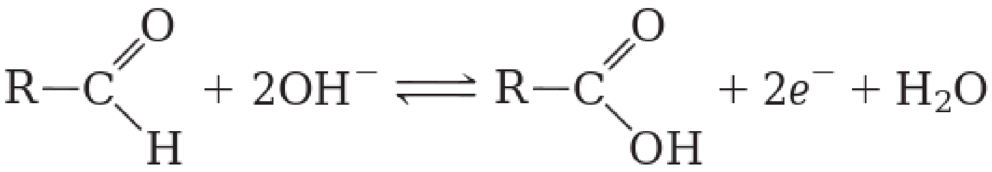
Cu^{2+} - oxidizing agend, oxidant

Fe^{2+}/Fe3^{+} - conjugate redox pair



$$\mathrm{R{-}C({=}O)H} + 4\mathrm{OH}^- + 2\mathrm{Cu}^{2+} \rightleftharpoons \mathrm{R{-}C({=}O)OH} + \mathrm{Cu_2O} + 2\mathrm{H_2O}$$

(1)



$$\mathrm{R{-}C({=}O)H} + 2\mathrm{OH}^- \rightleftharpoons \mathrm{R{-}C({=}O)OH} + 2e^- + \mathrm{H_2O}$$

(2) $2\mathrm{Cu}^{2+} + 2e^{-} + 2\mathrm{OH}^{-} \rightleftharpoons \mathrm{Cu_2O} + \mathrm{H_2O}$

DEHYDROGENATION

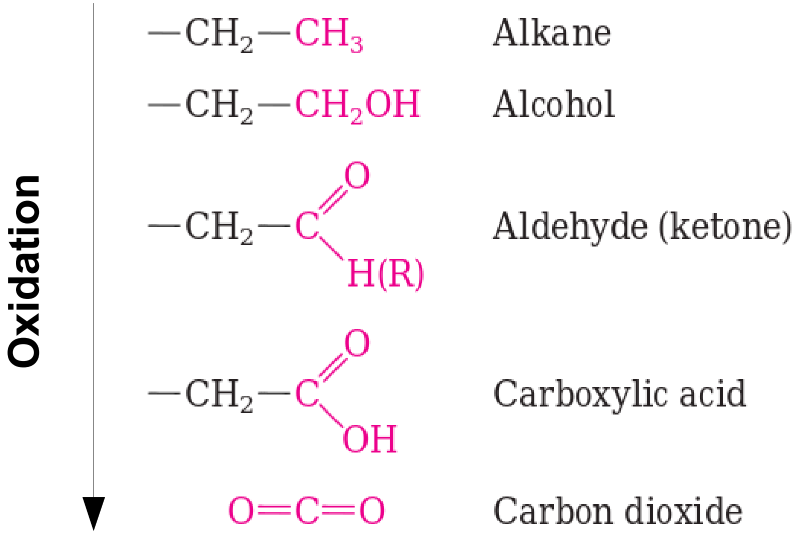
Electronegativity: H < C < S < N < O

Oxidation ~ dehydrogenation

Electron transfer:

- **Direct: $Fe^{2+} + Cu^{2+} \Leftrightarrow Fe^{3+} + Cu^{+}$**
- **As hydrogen atoms: $AH_2 \Leftrightarrow A + 2e^{-} + 2H^{+}$**
- **As hydride ion ($:H^{-}$)**
- **Combination with oxygen:**

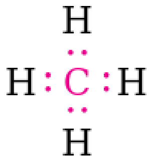
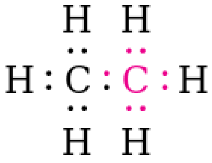
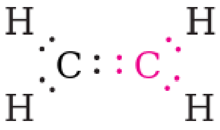
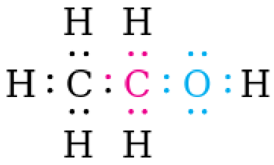
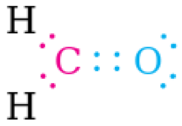
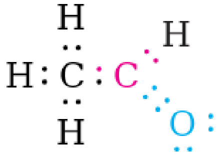
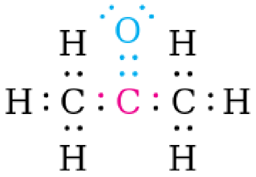
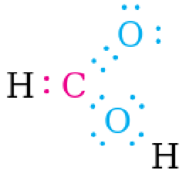
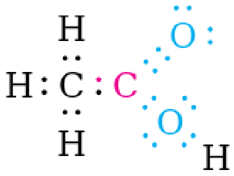
$R\text{-}CH_3 + 1/2O_2 \Leftrightarrow R\text{-}CH_2\text{-}OH$



Reducing equivalent

1/2 Unit of biological oxidation

CARBON OXIDATION STATES

Methane		8
Ethane (alkane)		7
Ethene (alkene)		6
Ethanol (alcohol)		5
Acetylene (alkyne)	H : C : : : C : H	5
Formaldehyde		4
Acetaldehyde (aldehyde)		3
Acetone (ketone)		2
Formic acid (carboxylic acid)		2
Carbon monoxide	: C : : : O :	2
Acetic acid (carboxylic acid)		1
Carbon dioxide		0

REDUCTION POTENTIAL

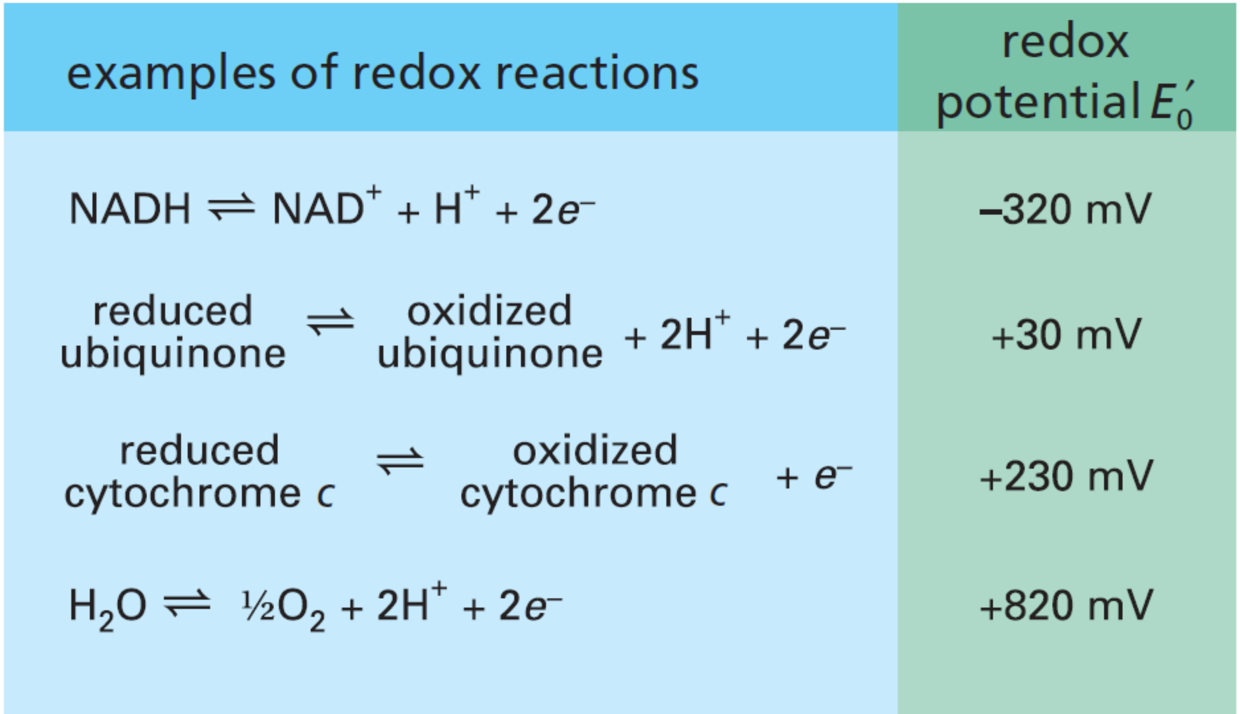
Reduction potential E^0 describes affinitity of the electron.

$H^+ + e^- <=> 1/2H_2$- reference standard 0.00 V)

$E = E^0 + RT/nF \ln([\text{electron acceptor}]/[\text{electron donor}])$

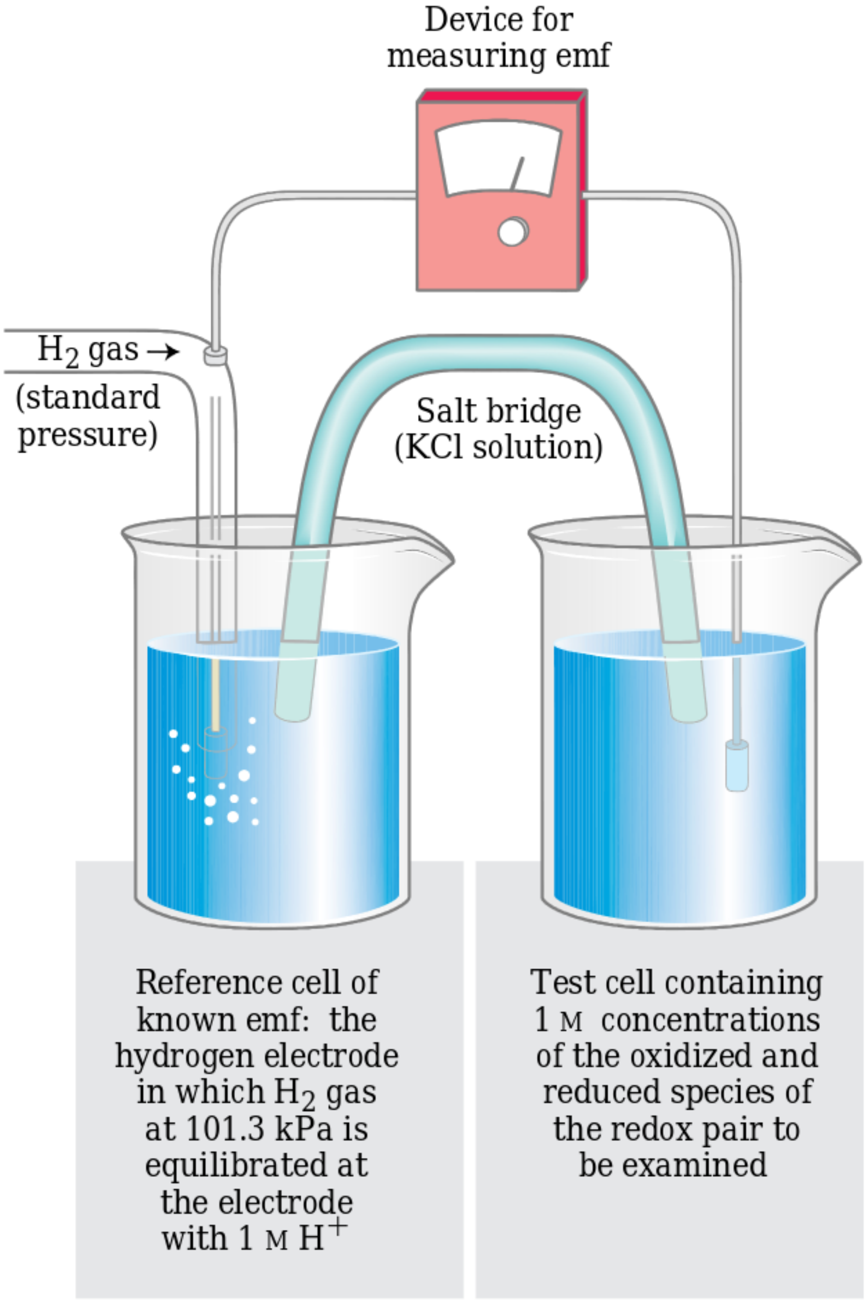
F- Faradays's constant 9.649×10^4, ($C\ mol^{-1}$); n- number of electrons

$\Delta G = -nF\ \Delta E$

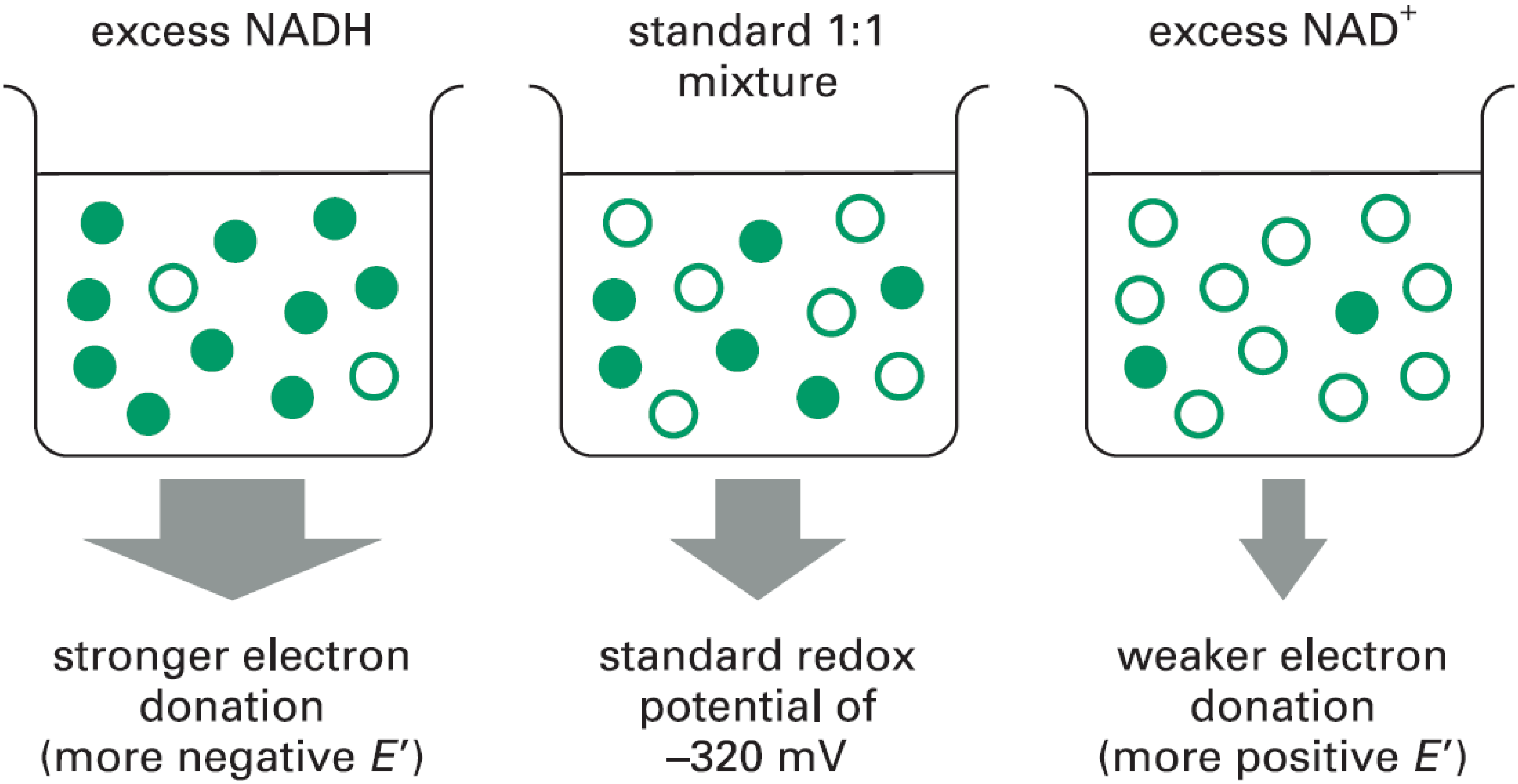


examples of redox reactions	redox potential E_0'
$NADH \rightleftharpoons NAD^+ + H^+ + 2e^-$	–320 mV
reduced ubiquinone $\rightleftharpoons$ oxidized ubiquinone $+ 2H^+ + 2e^-$	+30 mV
reduced cytochrome *c* $\rightleftharpoons$ oxidized cytochrome *c* $+ e^-$	+230 mV
$H_2O \rightleftharpoons \frac{1}{2}O_2 + 2H^+ + 2e^-$	+820 mV

REDUCTION POTENTIAL MEASUREMENTS



REDUCTION POTENTIAL MEASUREMENTS: CONCENTRATION DEPENDENCE



UNIVERSAL ELECTRON CARRIERS

- **Water-soluble co-enzymes:**

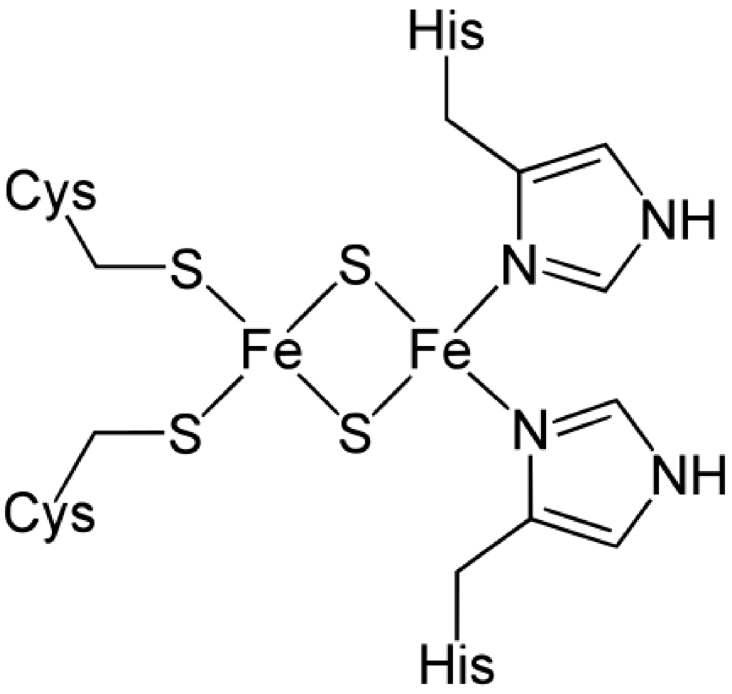
  - NAD^+/$NADP^+$; NAD/NADH non-covalently bound to enzymes

  - FMN, FAD covalently bound to enzymes

- **Quinones (lipid-soluble)**

- **Iron-sulfur proteins**

- **Cytochromes**



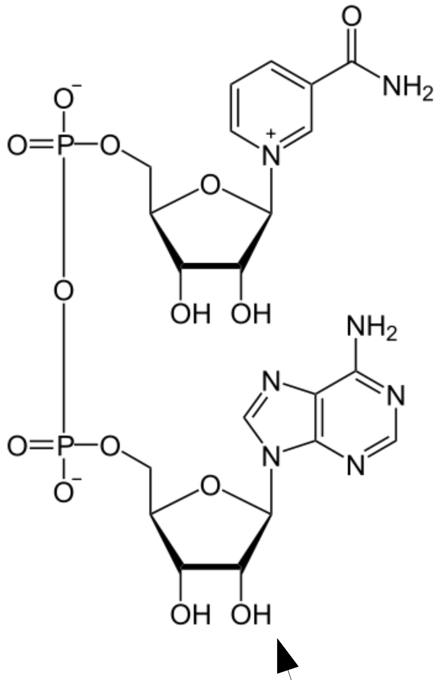
NADH/NADPH

- Nicotinamide adenine dinucleotide
- Pyridine nucleotides
- $NAD + 2e^- + 2H^+ \Rightarrow NADH + H^+$

  $NADP + 2e^- + 2H^+ \Rightarrow NADH + H^+$
- NAD is mostly reduced (used in oxidation), mitochondria

  $AH_2 + NAD^+ \Rightarrow A + NADH + H^+$
- NADP is mostly oxidized (used in reduction), cytosole

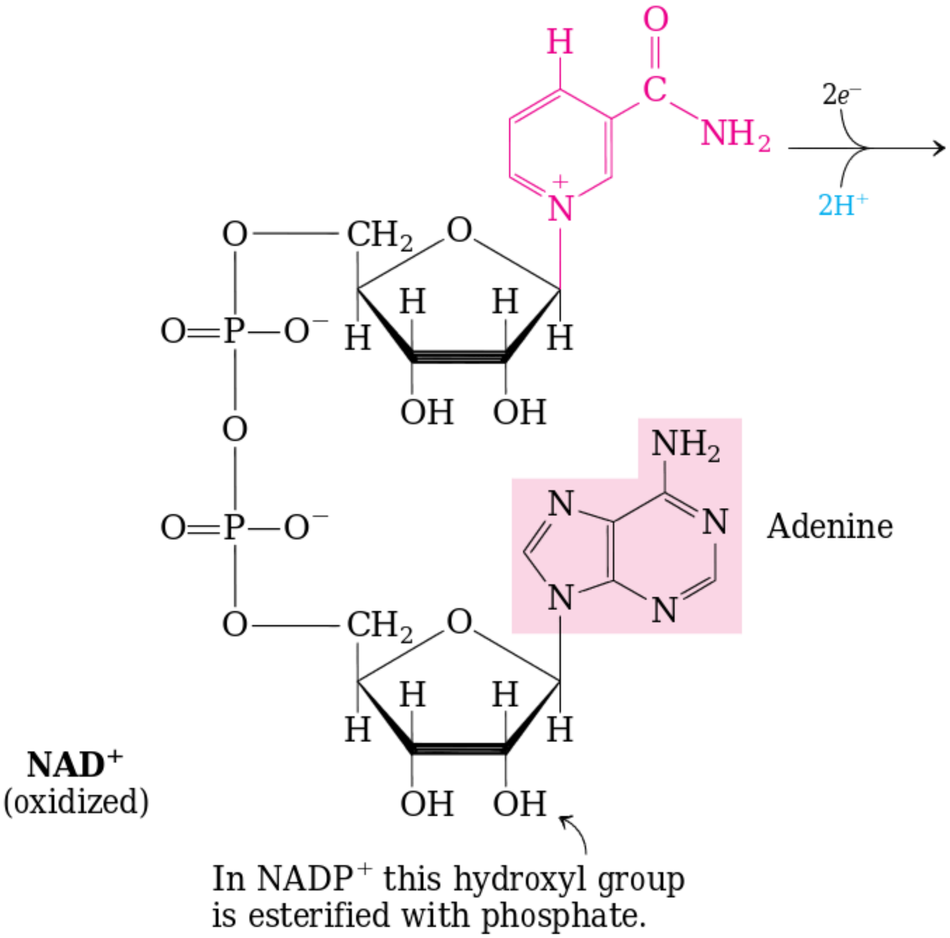
  $A + NADPH + H^+ \Rightarrow AH_2 + NADP^+$
- Oxidoreductases/dehydrogenases



NAD^+

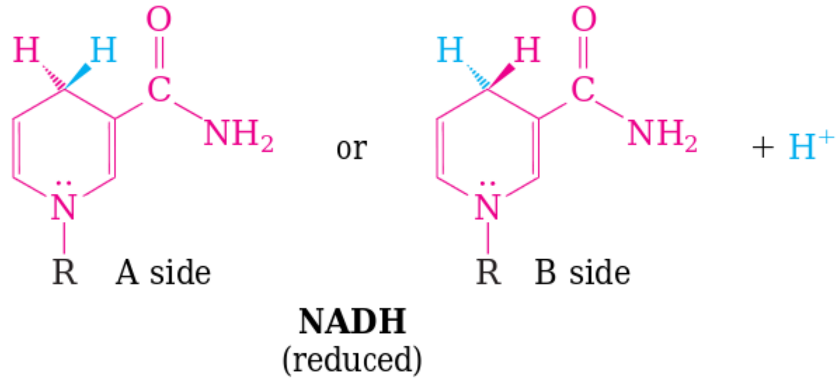
$NADP^+$: phosphorylated

NADH/NADPH

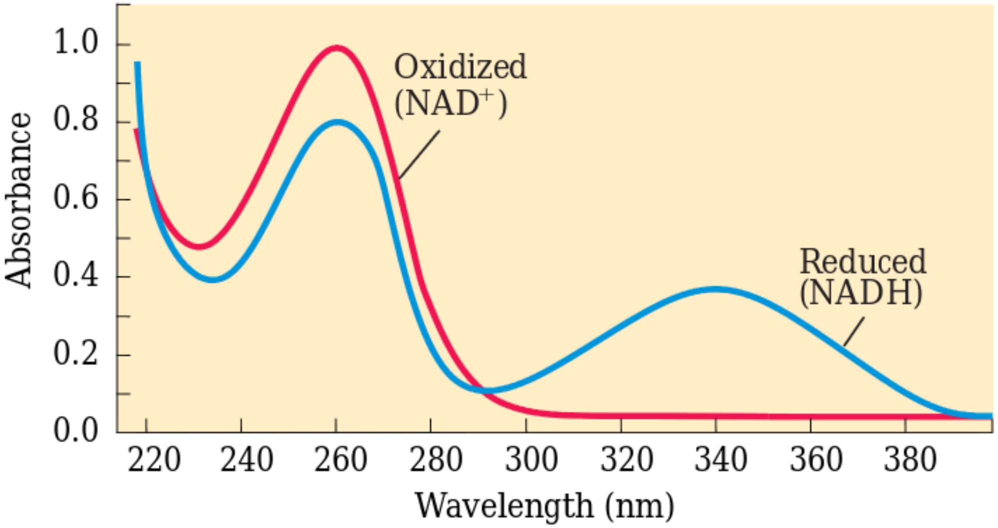


NAD$^+$
(oxidized)

In NADP$^+$ this hydroxyl group is esterified with phosphate.



NADH
(reduced)



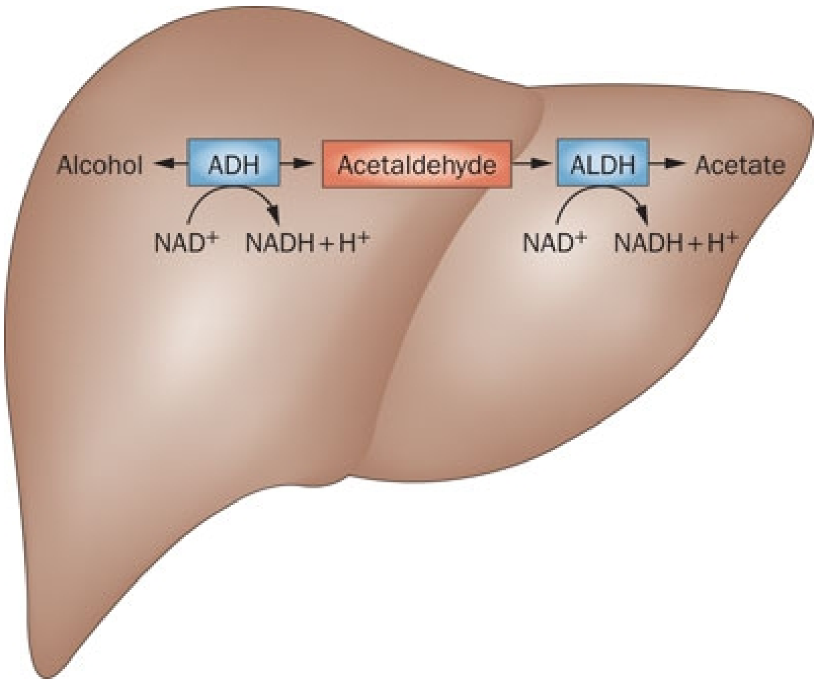
Specificity for A/B-sites (up to 7 orders)

NADH/NADPH: EXAMPLES

Alcohol dehydrogenase:

$CH_3CH_2OH + NAD^+ \Rightarrow CH_3CHO + NADH + H^+$

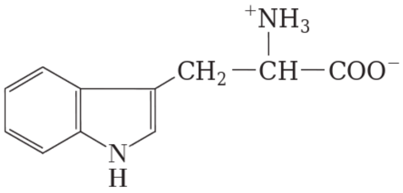
Ethanol ***Acetaldehyde***



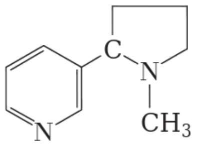
Combination of several dyhedrogenases (in fermentation):

$$
\begin{aligned}
&(1)\quad \text{Glyceraldehyde 3-phosphate} + \text{NAD}^+ \longrightarrow \\
&\qquad\qquad\qquad\qquad \text{3-phosphoglycerate} + \text{NADH} + \text{H}^+ \\
&(2)\quad \text{Acetaldehyde} + \text{NADH} + \text{H}^+ \longrightarrow \text{ethanol} + \text{NAD}^+ \\
\hline
&\textit{Sum:}\quad \text{Glyceraldehyde 3-phosphate} + \text{acetaldehyde} \longrightarrow \\
&\qquad\qquad\qquad\qquad \text{3-phosphoglycerate} + \text{ethanol}
\end{aligned}
$$

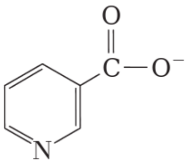
NAD/NADP SOURCES



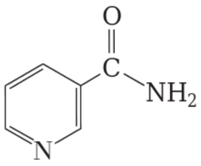
Tryptophan



Nicotine

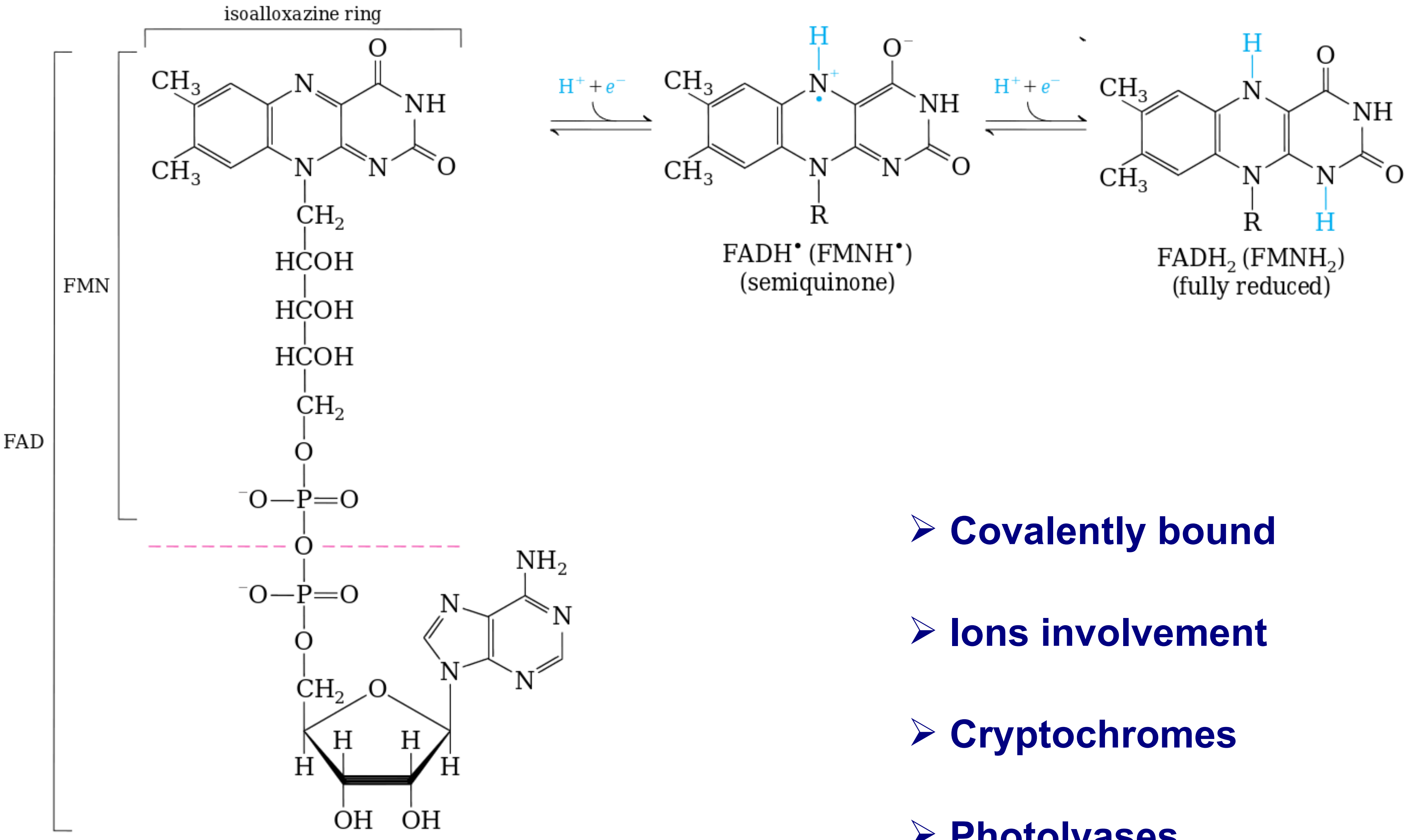


Niacin
(nicotinic acid)



Nicotinamide

FLAVOPROTEINS: FMN, FAD

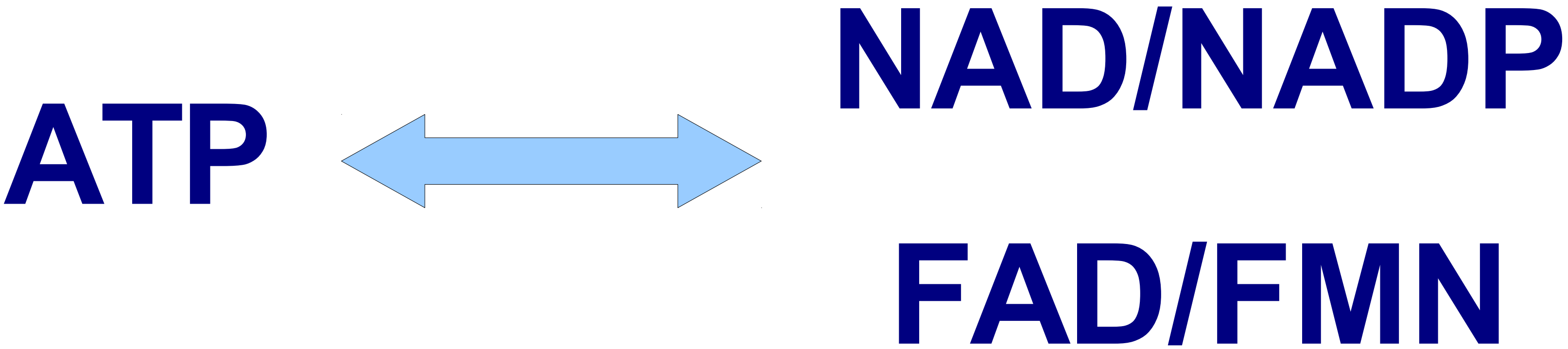


Flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN)

- ➢ **Covalently bound**
- ➢ **Ions involvement**
- ➢ **Cryptochromes**
- ➢ **Photolyases**

PHOSPHORYLATION vs. ELECTRON TRANSFER

Coupling of phosphorylation with electron transfer is fundamental for metabolism.



ATP

NAD/NADP

FAD/FMN

PHOTOSYNTHESIS

Photosynthesis: convertion of light energy into chemical energy.

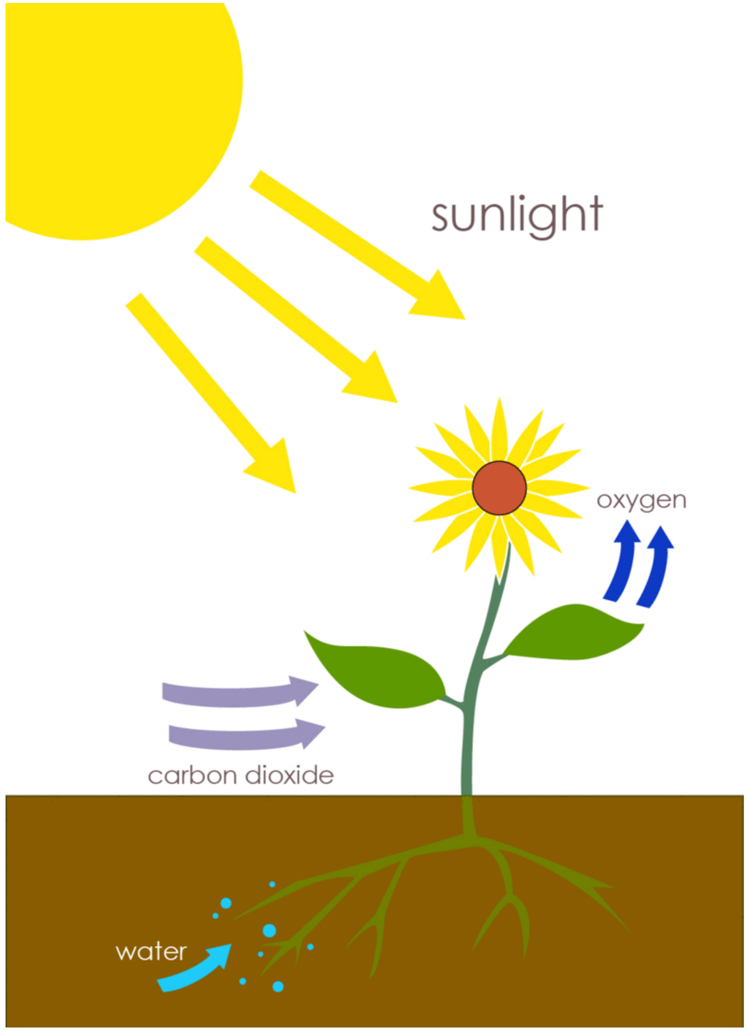
$6CO_2 + 6H_2O \Rightarrow C_6H_{12}O_6 + 6O_2$

Photosynthetic organisms:

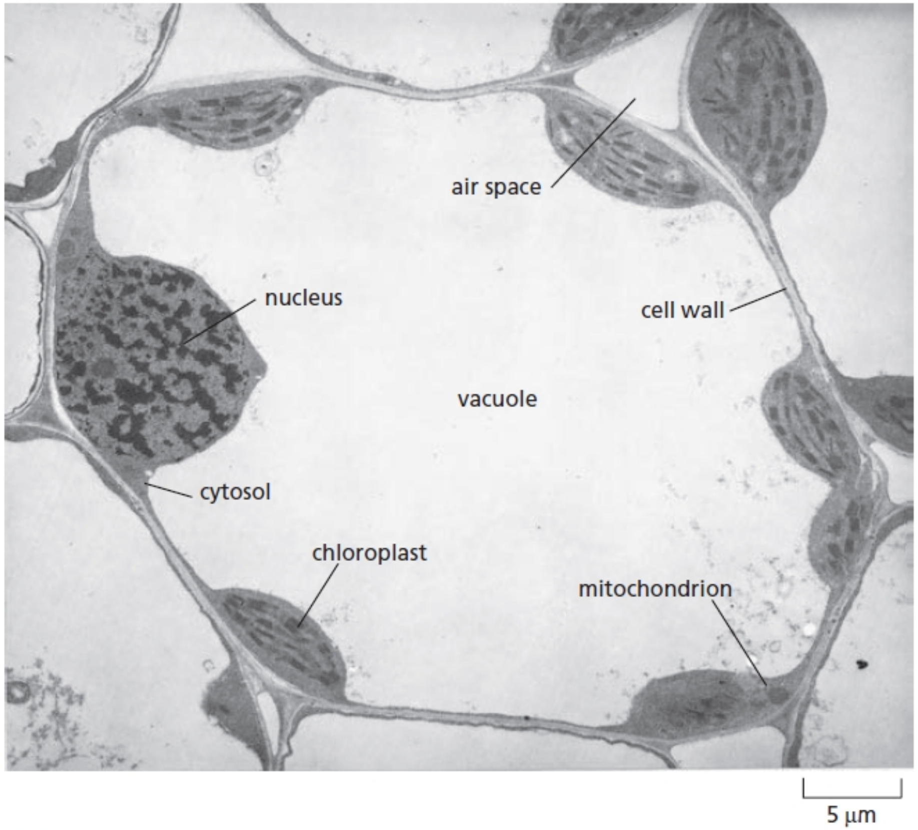
- **Cyanobacteria**
- **Algae**
- **Plants**

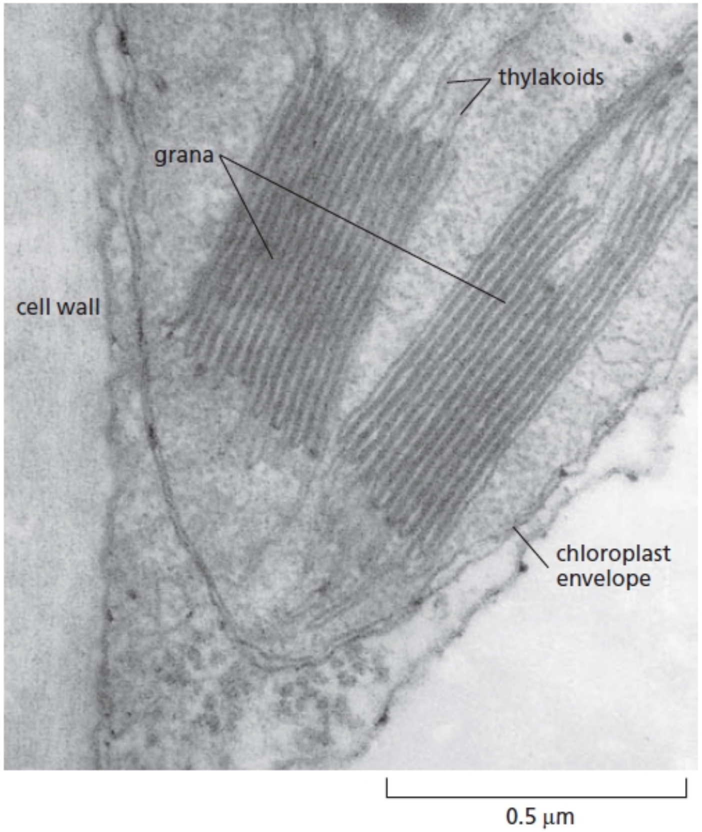
Endocytosis

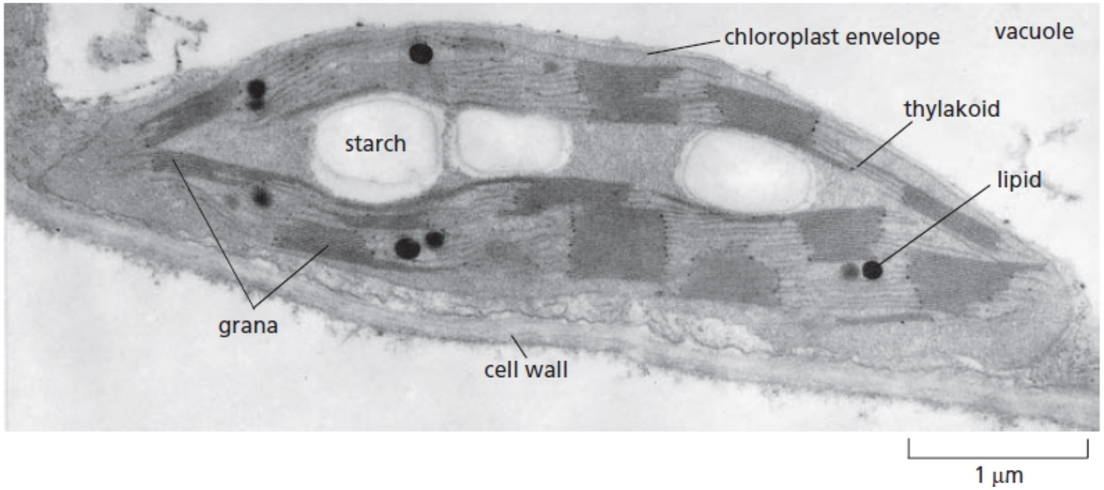
chloroplasts



CHLOROPLASTS

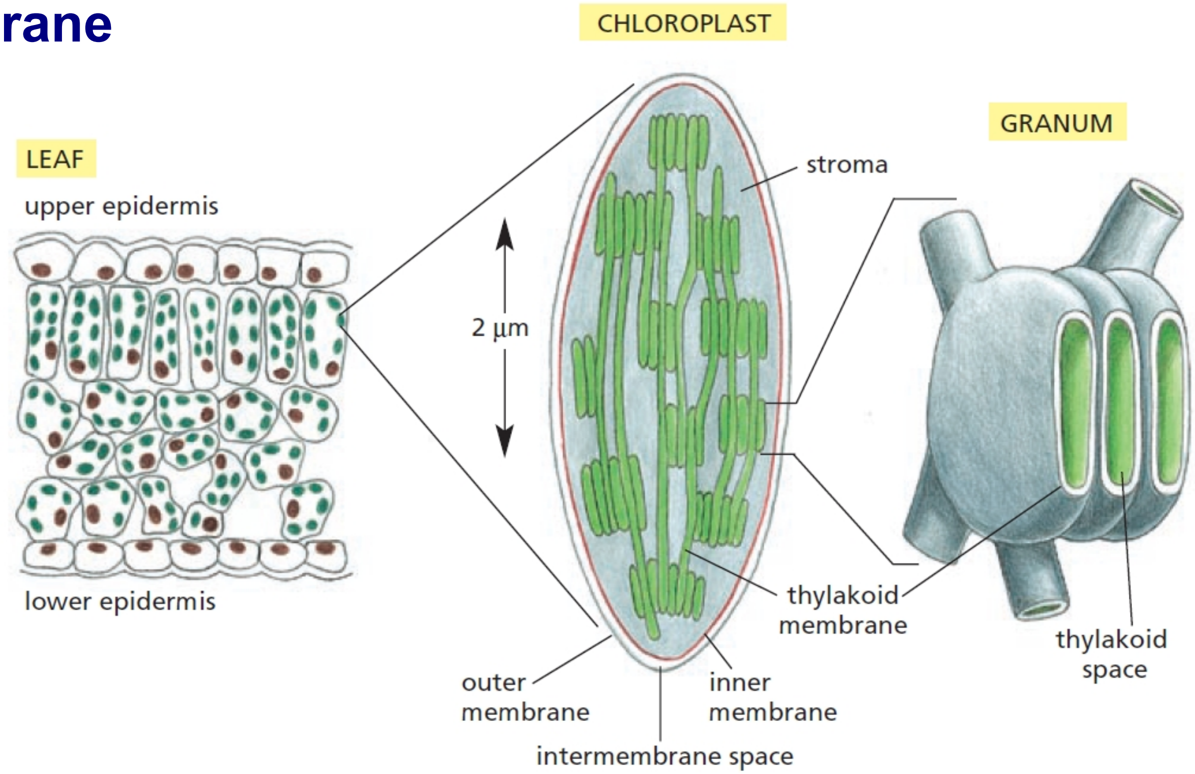






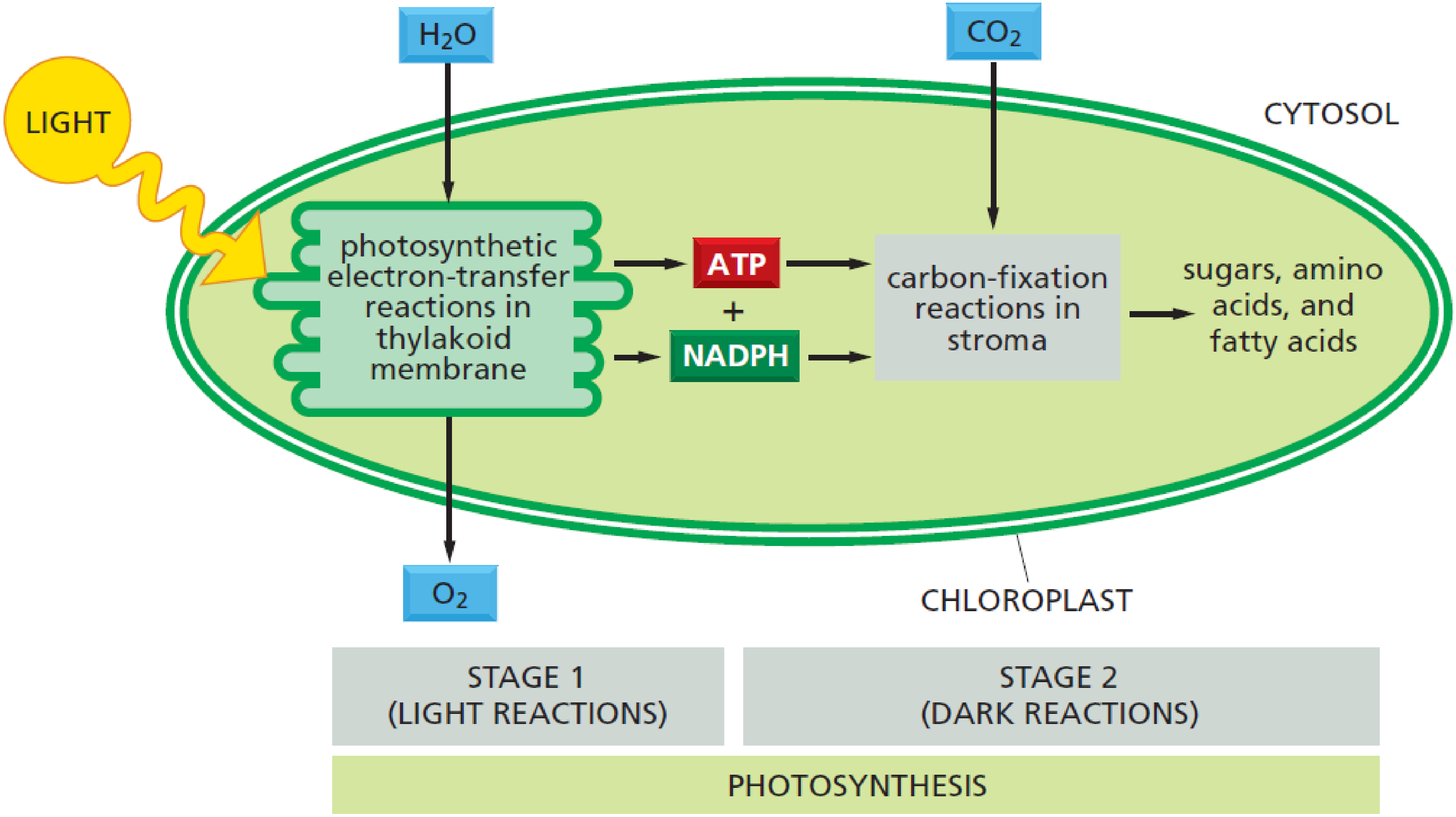
CHLOROPLASTS

- Plastid family of organelles
- Own small genome (circular DNA, 100-200 kb, up to 200 mRNA)
- Two concentric membranes: outer is highly permeable, inner forms stroma
- Proplastides => etioplasts (no light), chloroplasts
- Leucoplasts (f.i. amyloplast): storage function
- Thylakiod membrane



REACTIONS IN CHLOROPLASTS

- **Photosynthetic electron-transfer**
- **Carbon-fixation reactions**

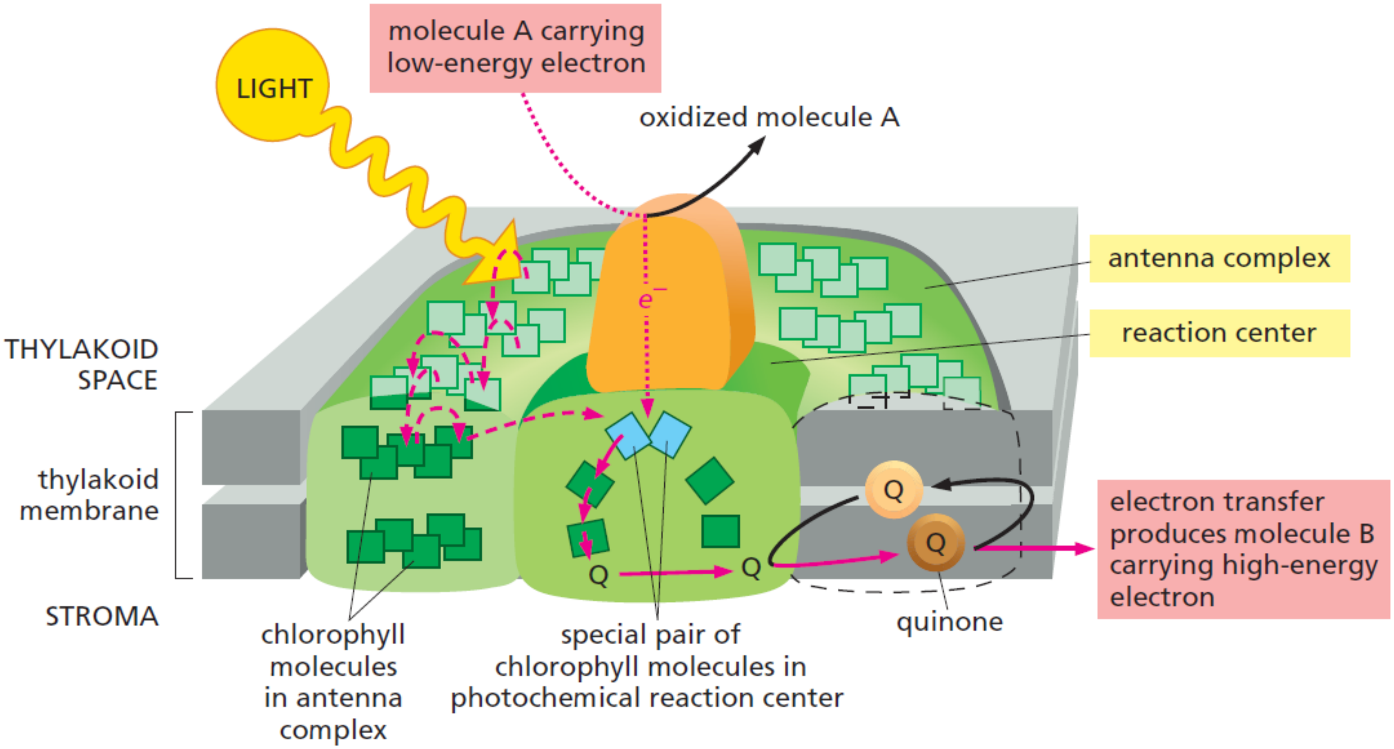


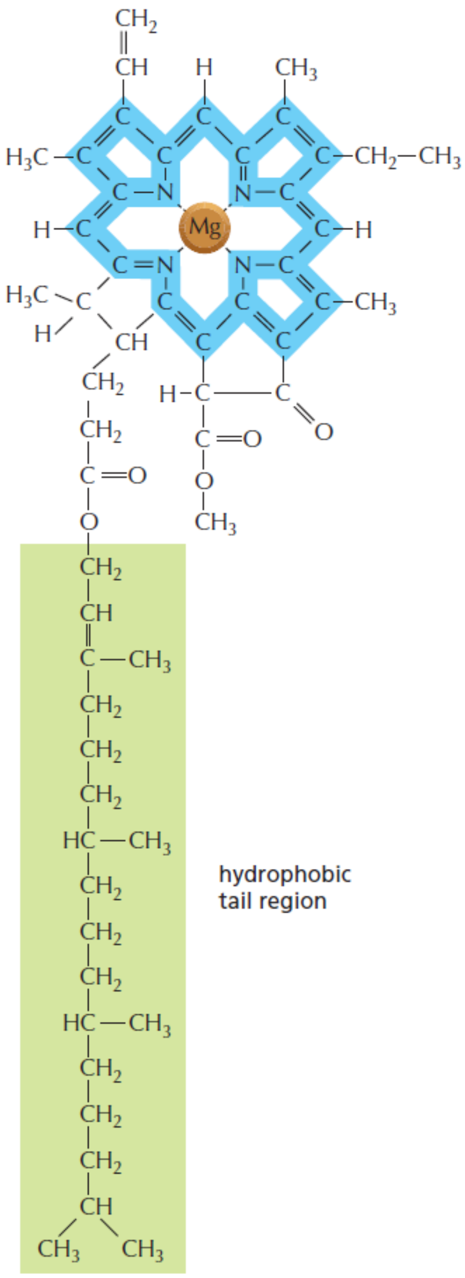
REACTIONS IN CHLOROPLASTS

- Photosynthetic electron-transfer
  - An electron from chlorophyll (induce by the light) => e-transfer chain
  - O_2 is produced, H^+ is pumped from thylakoid membrane, APT is synthetized
  - Electrons are loaded onto $NADP^+$ => NADPH
- Carbon-fixation reactions
  - NADPH and ATP supply the energy for CO_2 => carbohydrate (sucrose)
  - Reactions begins in the stroma and then continues in the cytosole
  - Sucrose is transferred further to other tissues

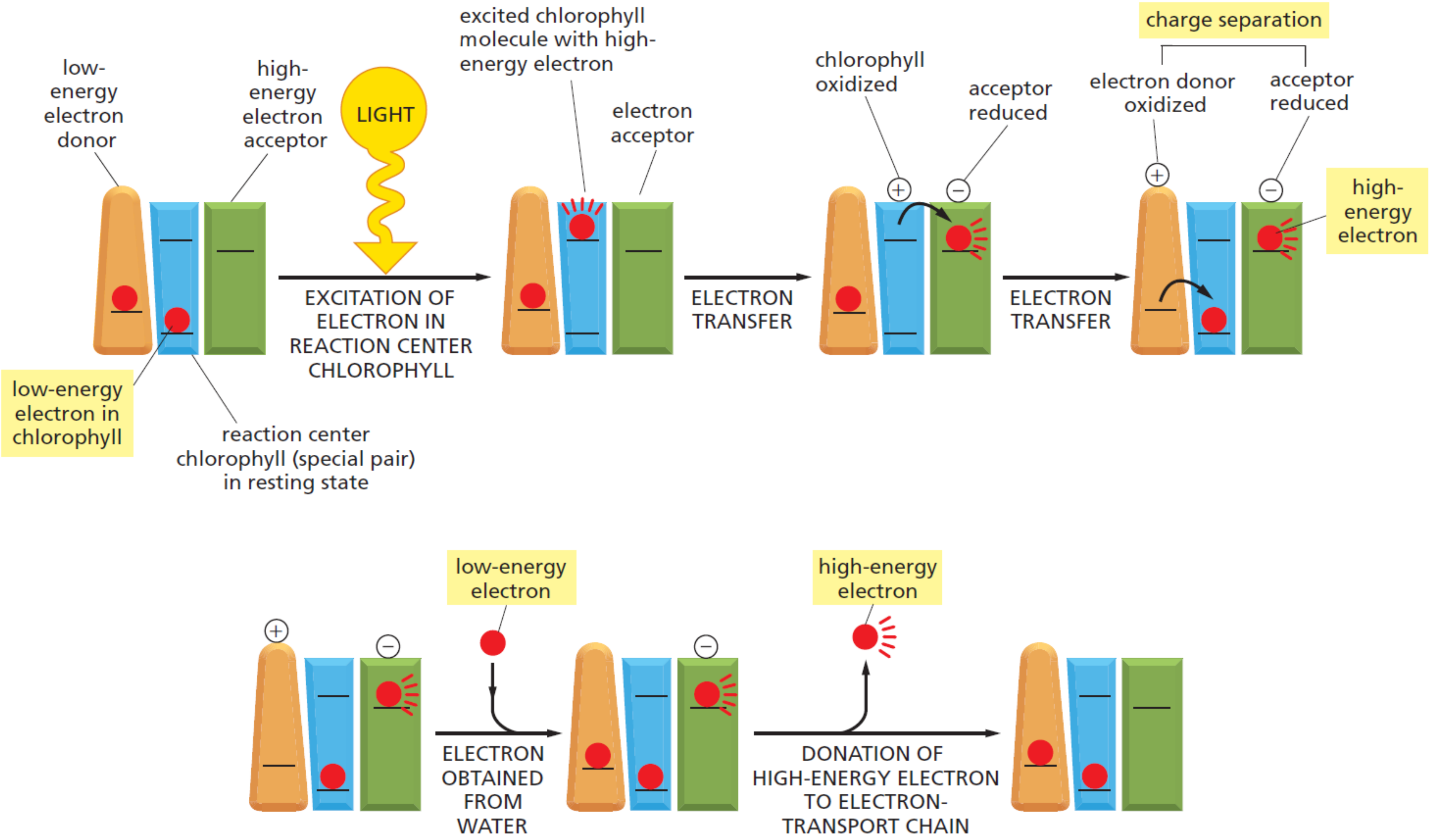
ABSORBTION OF THE SUNLIGHT BY CHLOROPHYLL

- Photoeffect occurs in chlorophyll
- Chlorophyll is a part of the photosystem
- Photosystem includes antenna complex and a reaction center
- Electron-transfer goes up to special pairs





PRINCIPLE OF CHARGE SEPARATION



low-energy electron donor

high-energy electron acceptor

LIGHT

low-energy electron in chlorophyll

reaction center chlorophyll (special pair) in resting state

EXCITATION OF ELECTRON IN REACTION CENTER CHLOROPHYLL

excited chlorophyll molecule with high-energy electron

electron acceptor

ELECTRON TRANSFER

chlorophyll oxidized

acceptor reduced

ELECTRON TRANSFER

charge separation

electron donor oxidized

acceptor reduced

high-energy electron

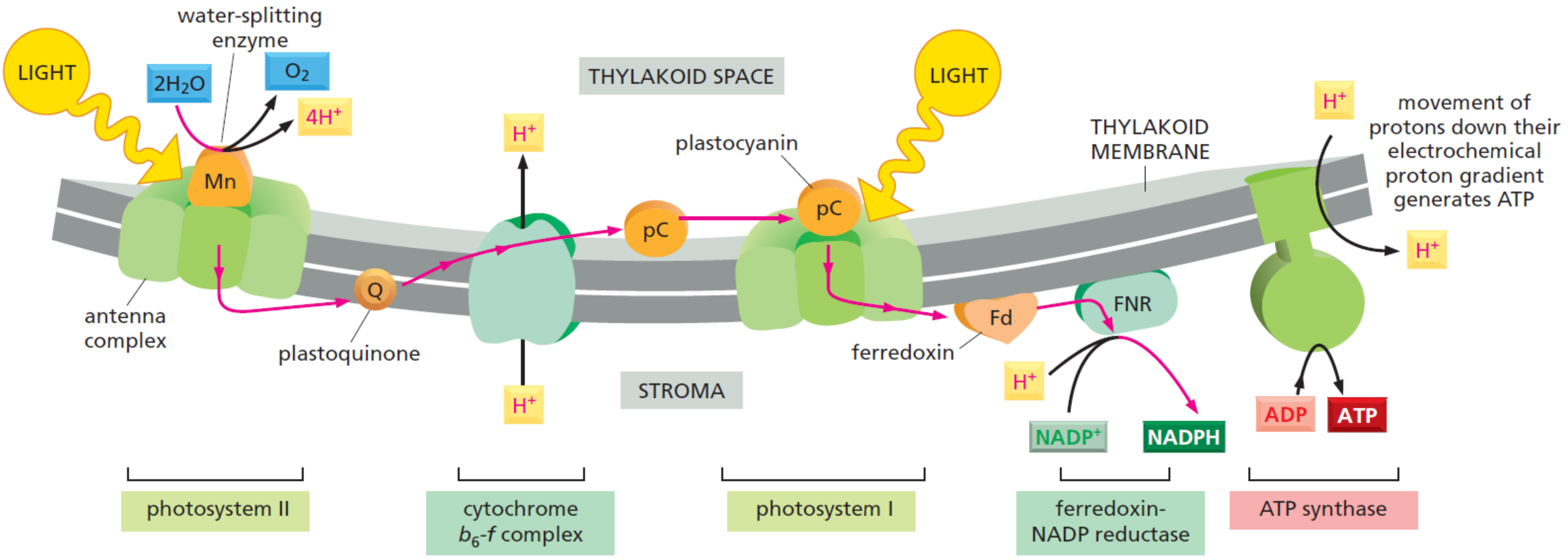
low-energy electron

ELECTRON OBTAINED FROM WATER

high-energy electron

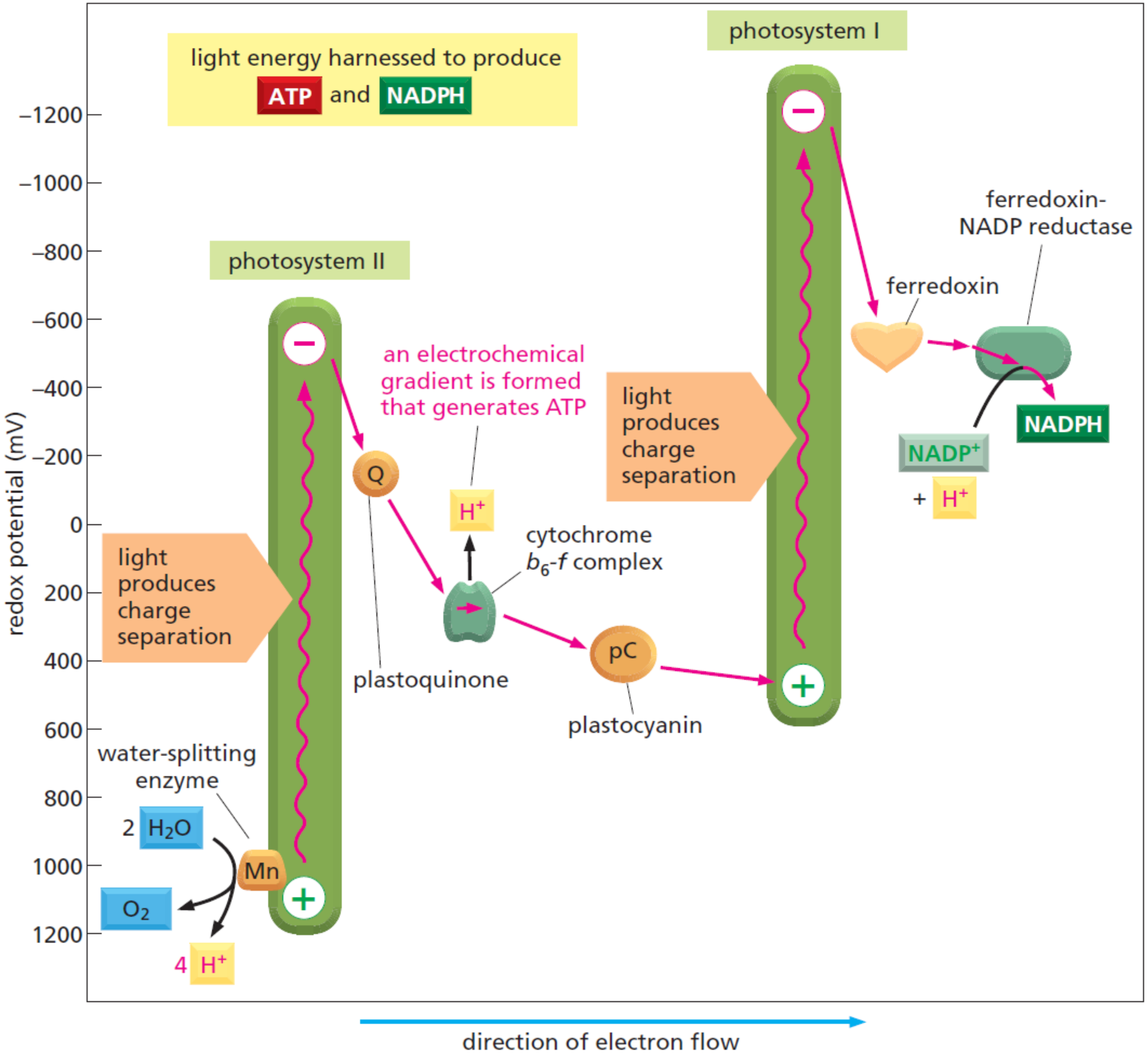
DONATION OF HIGH-ENERGY ELECTRON TO ELECTRON-TRANSPORT CHAIN

LIGHT ENERGY IS USED FOR SYNTHESIS OF ATP AND NADPH

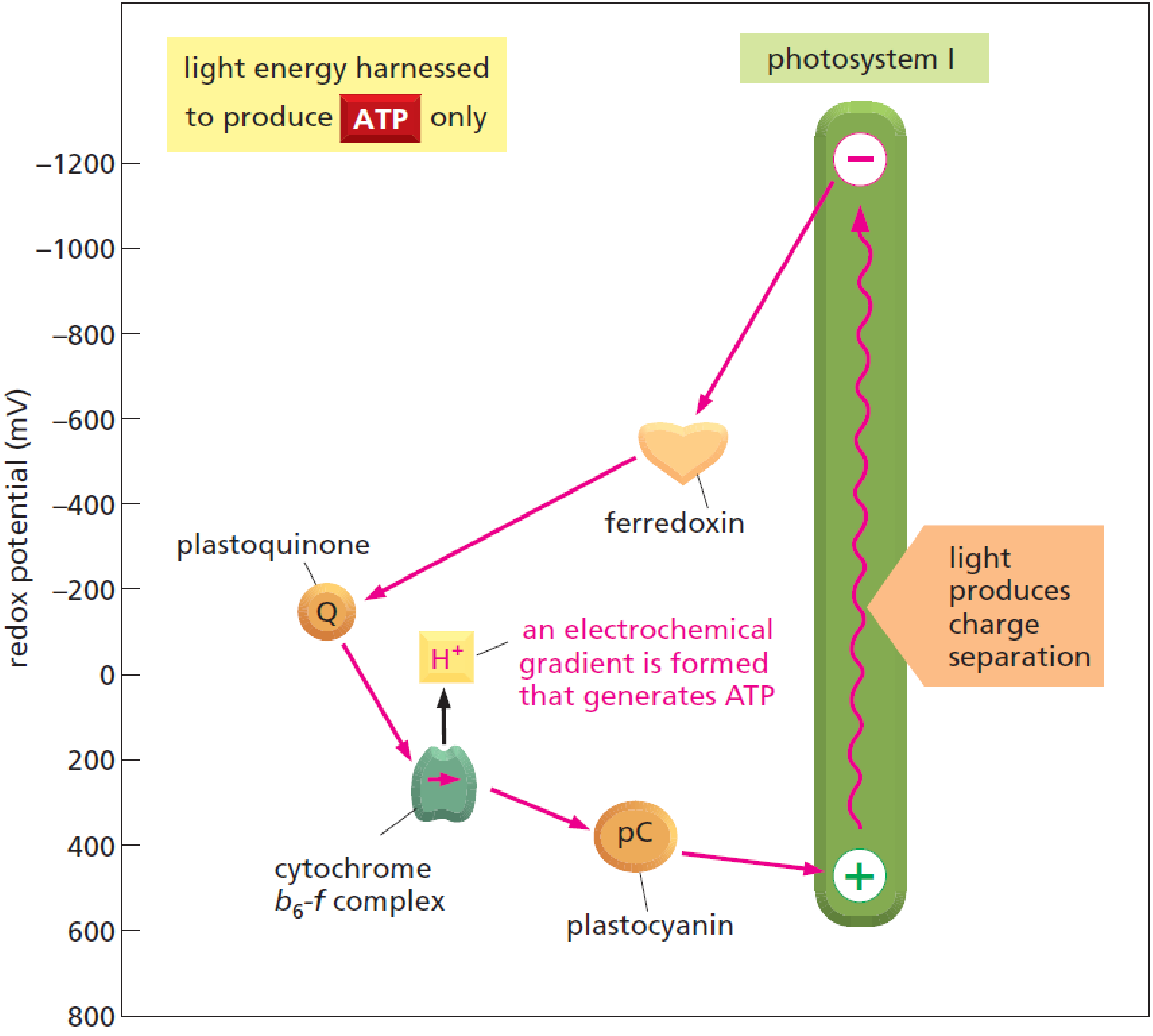


Mn-dependent water splitting enzyme in the photosystem II

CHANGE OF THE ELECTRON REDOX POTENTIAL DURING THE PHOTOSYNTHESIS

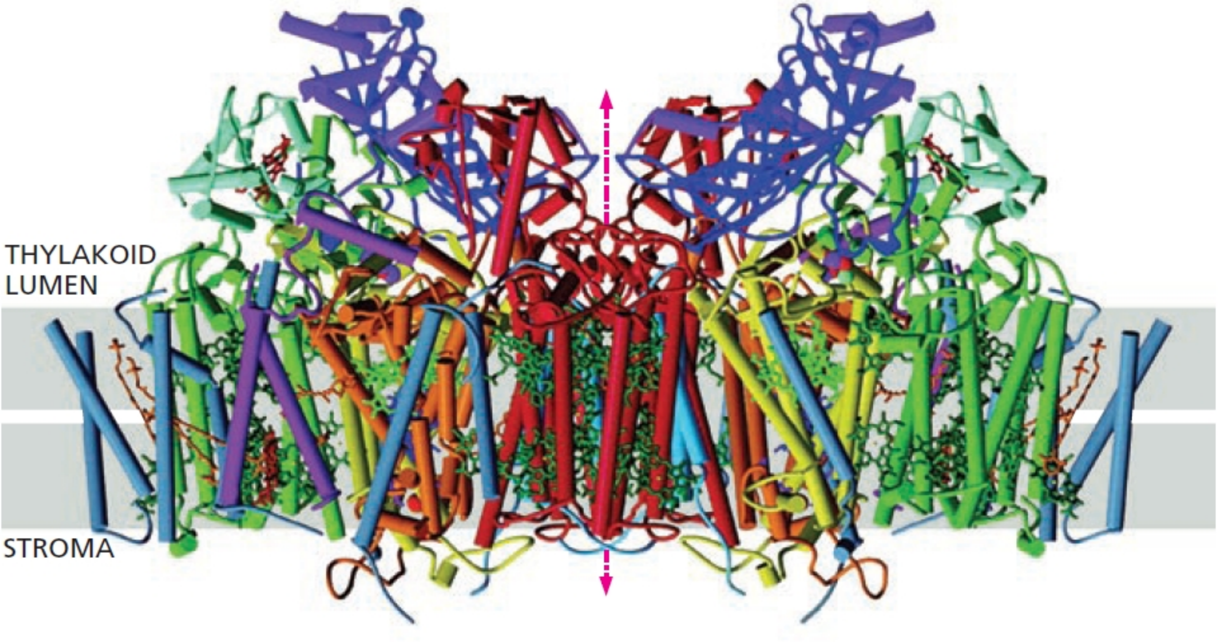


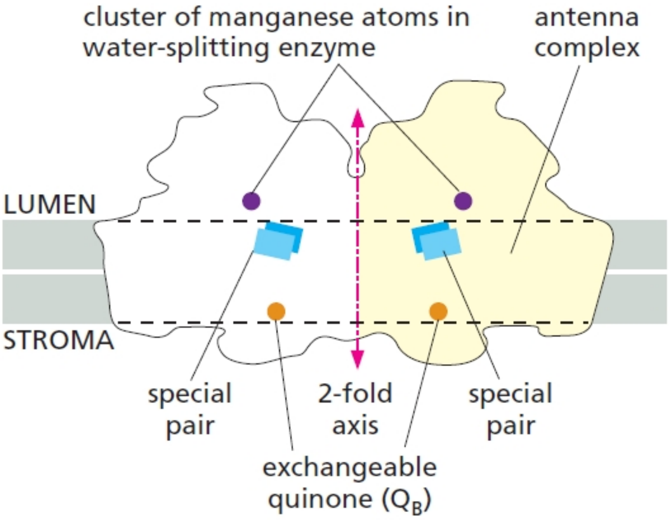
PHOTOSYSTEM I CAN SYNTHETIZE ONLY ATP



PHOTOSYSTEM STRUCTURES

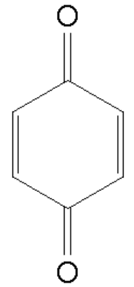
~ 20 proteins



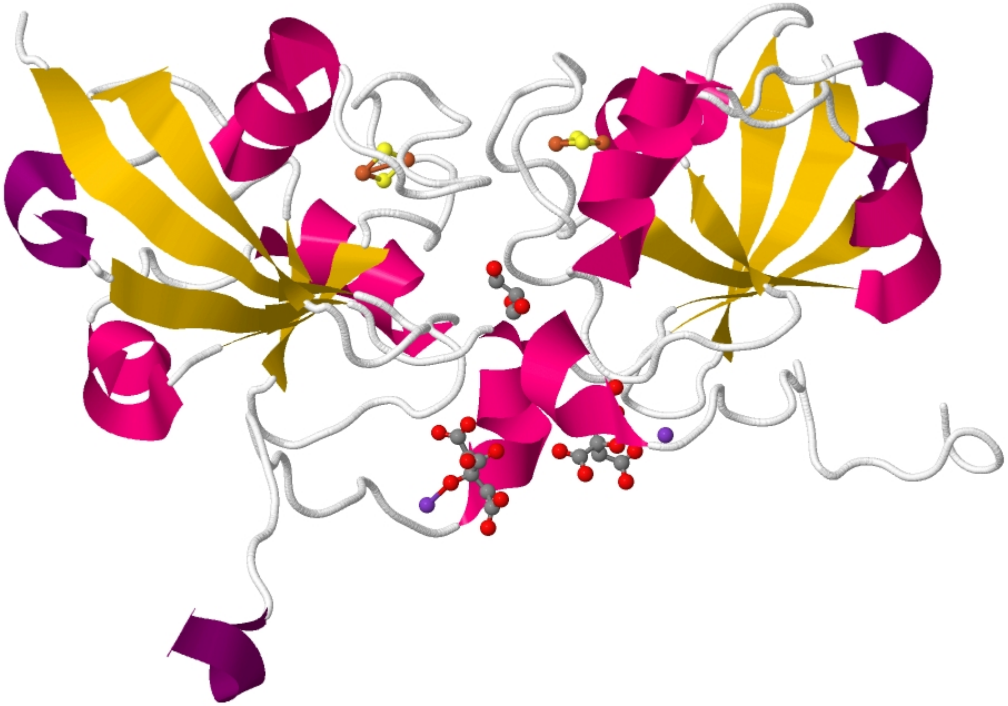


PHOTOSYSTEM PARTICIPANTS

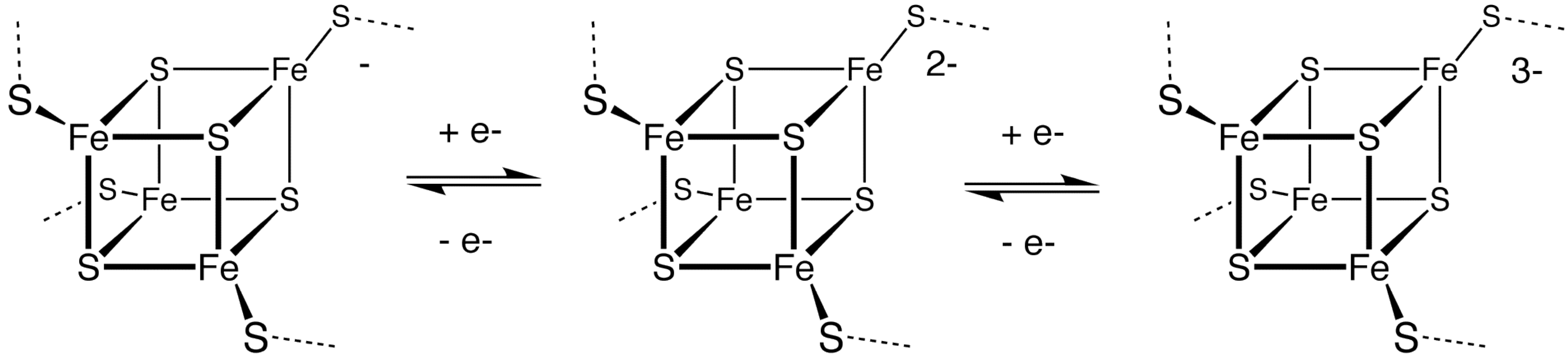
- **Plastoquinone**
- **Ferredoxin**



Quinone



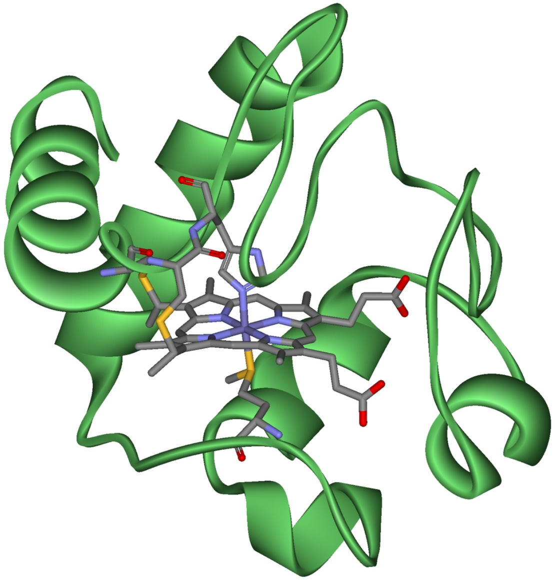
PDB ID: 3P1M

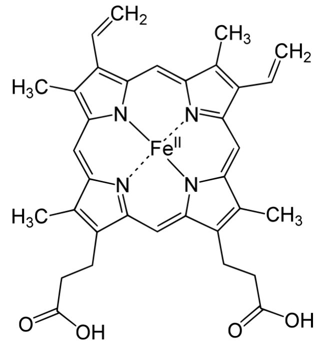


PHOTOSYSTEM PARTICIPANTS

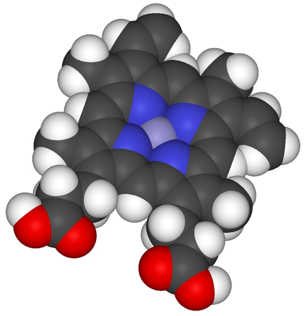
- **Cytochromes**

PDB ID: 1HRC



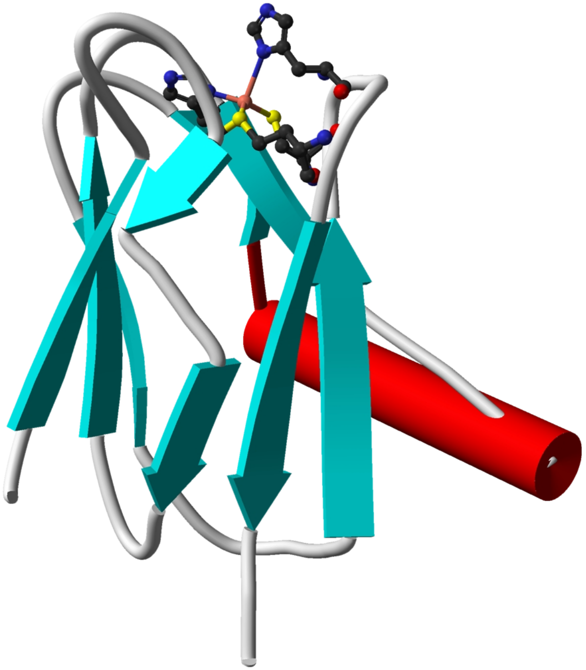


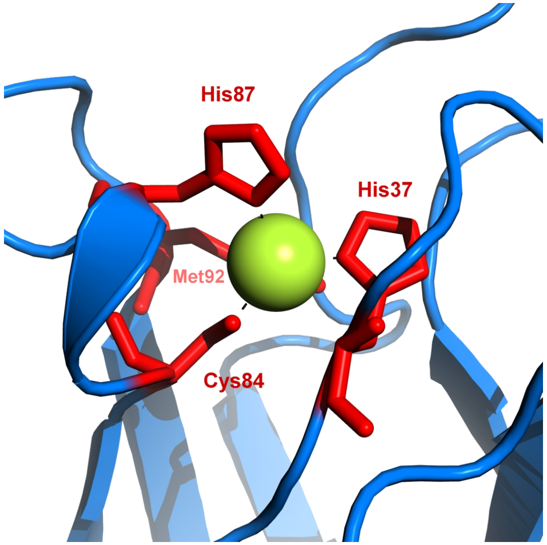
Heme



- **Plastocyanine**

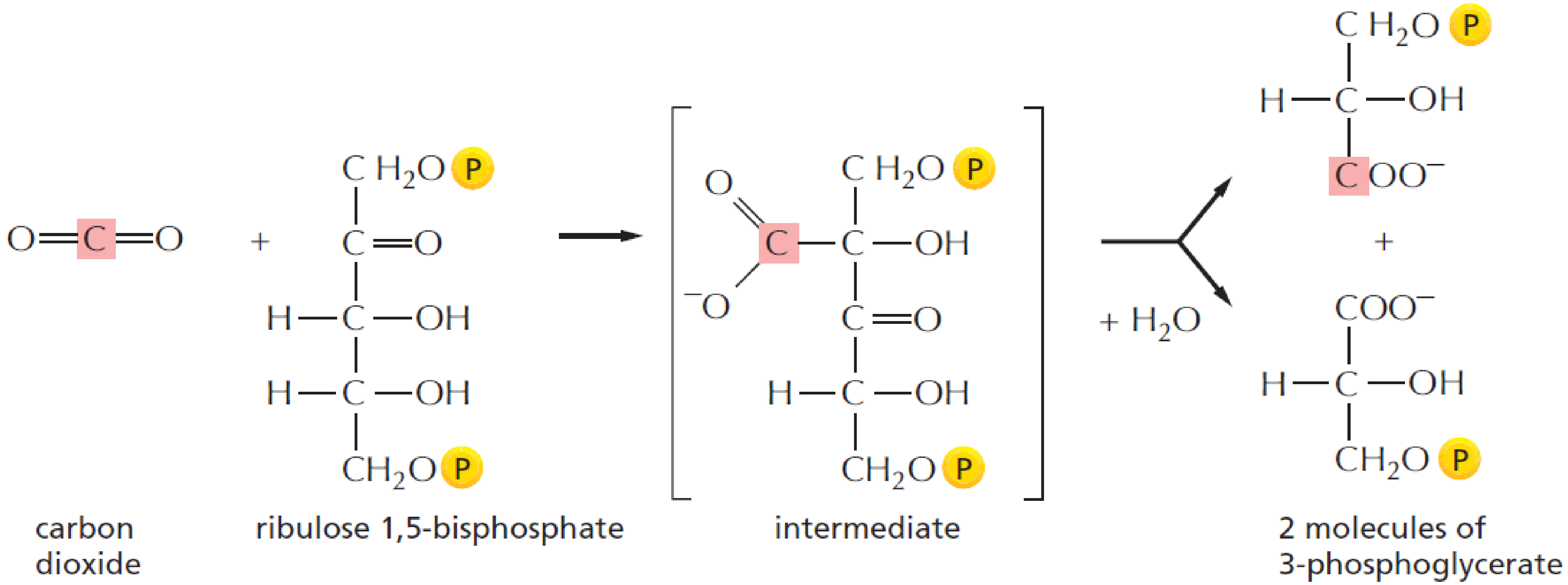
PDB ID: 3BQV



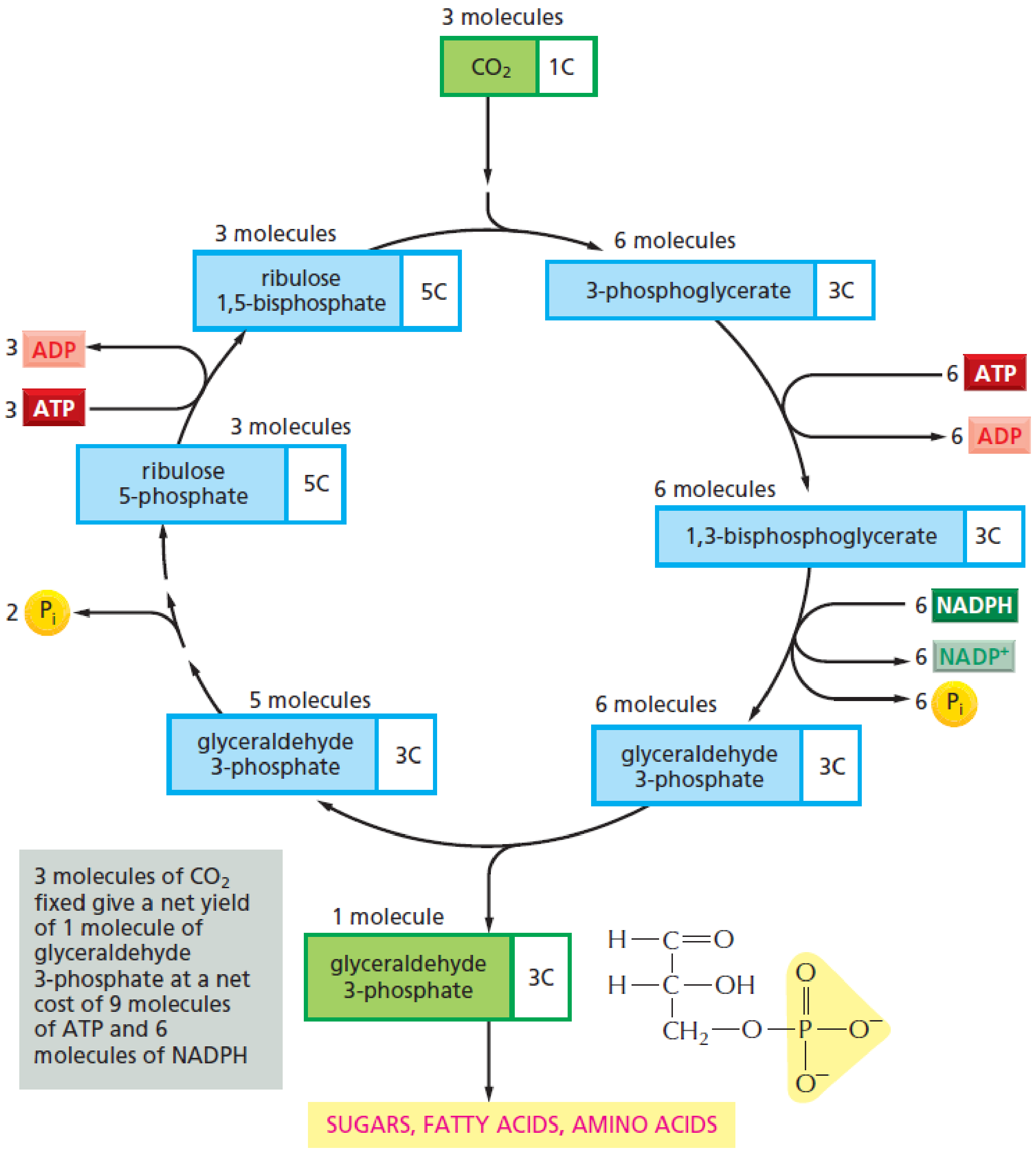


CARBON FIXATION USES ATP AND NADPH

- ATP and NADPH are in chloroplast stroma, which membrane is not permeable
- They are used for carbone fixation by ribulose bisphosphate carboxylase (~50% of total chloroplast proteins)



CARBON FIXATION CYCLE: CALVIN CYCLE

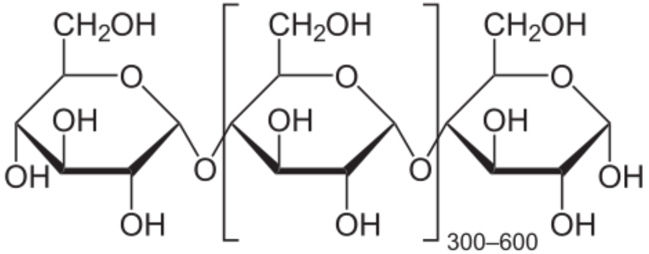


3 molecules of CO_2 fixed give a net yield of 1 molecule of glyceraldehyde 3-phosphate at a net cost of 9 molecules of ATP and 6 molecules of NADPH

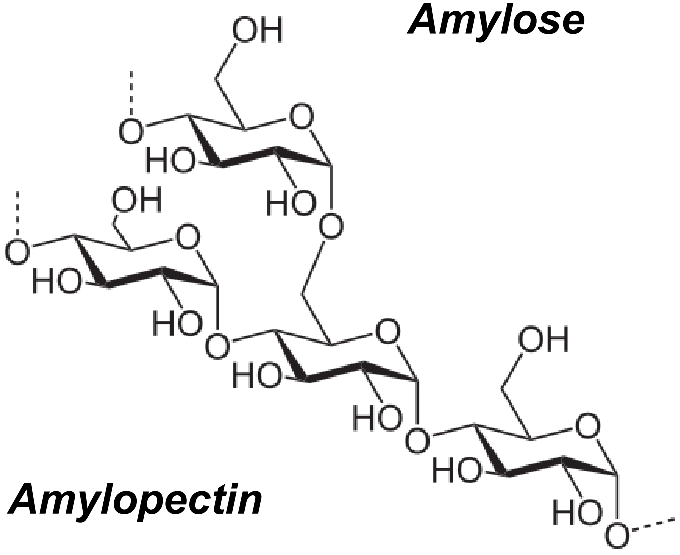
$$3CO_2 + 9ATP + 6NADPH + H_2O \Rightarrow \text{glyceraldehyde-3P} + 8P_i + 9ADP + 6NADP^+$$

GLYCERALDEHYDE-3P (G-3P)

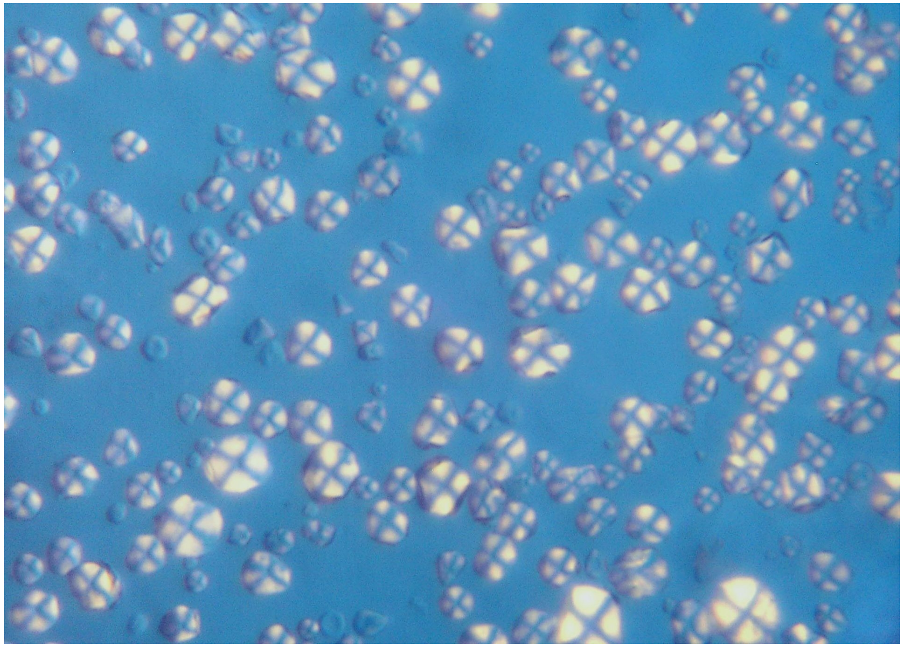
- G-3P can be transported to cytosole.
- G-3P is an intermediate for sucrose synthesis in glycolysis.
- G-3P partially remains in starch in stroma (carbohydrate depot).
- Carbohydrates from the starch are used in the dark phase.



Amylose



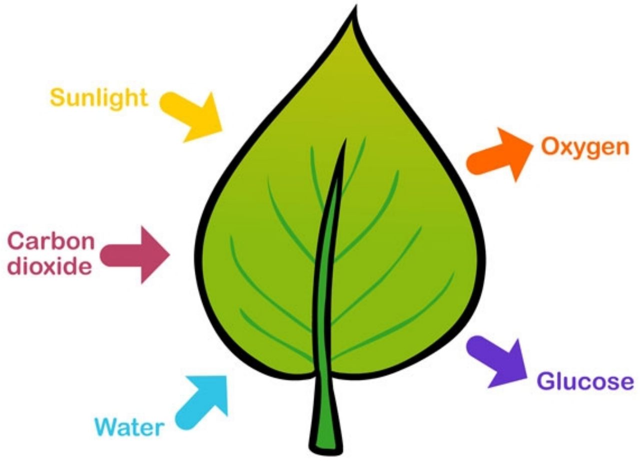
Amylopectin



Starch granules

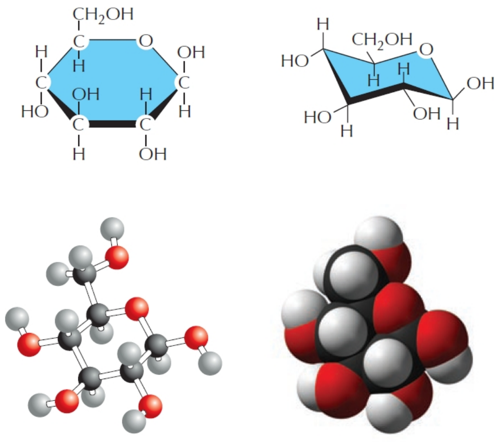
PHOTOSYNTHESIS: SUMMARY

- Light activates electrons in the chlorophyll of photosynthetic bacteria and chloroplasts in plants.
- Electron is transported along the electron chain to the reaction center.
- One (purple photosynthetic bacteria) or two (cyanobacteria, chloroplasts) photosystems produce NADPH, ATP, O_2.
- In stroma, NADPH and ATP are used to fix CO_2.
- The final product glyceraldehyde 3-phosphate can be transported to cytosole.

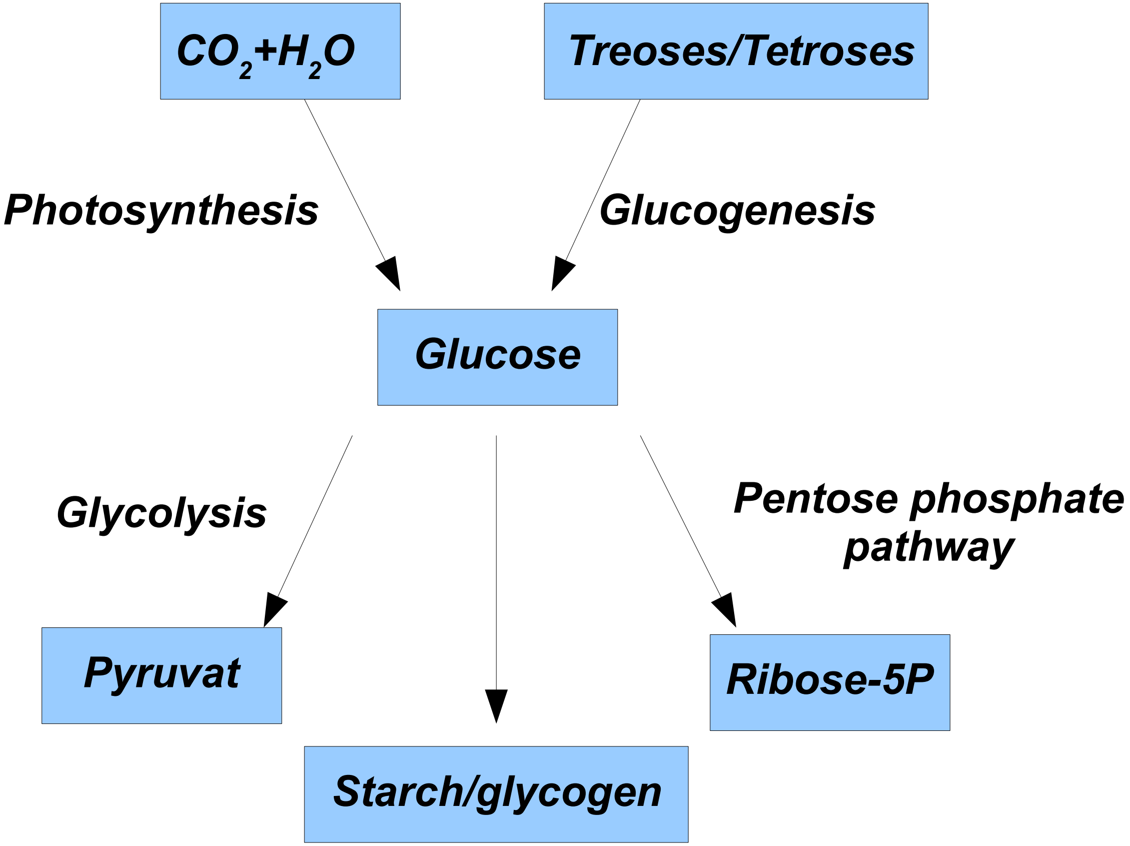


GLUCOSE

- **Glucose is a central metabolite.**
- **Full oxidation: -2.84 kJ/mol**
- **Sources: starch, glycogen**
- **Precursors: aa, nucleotides, coenzymes, fatty acids**



Glucose



CO_2+H_2O

Treoses/Tetroses

Photosynthesis

Glucogenesis

Glucose

Glycolysis

Pentose phosphate pathway

Pyruvat

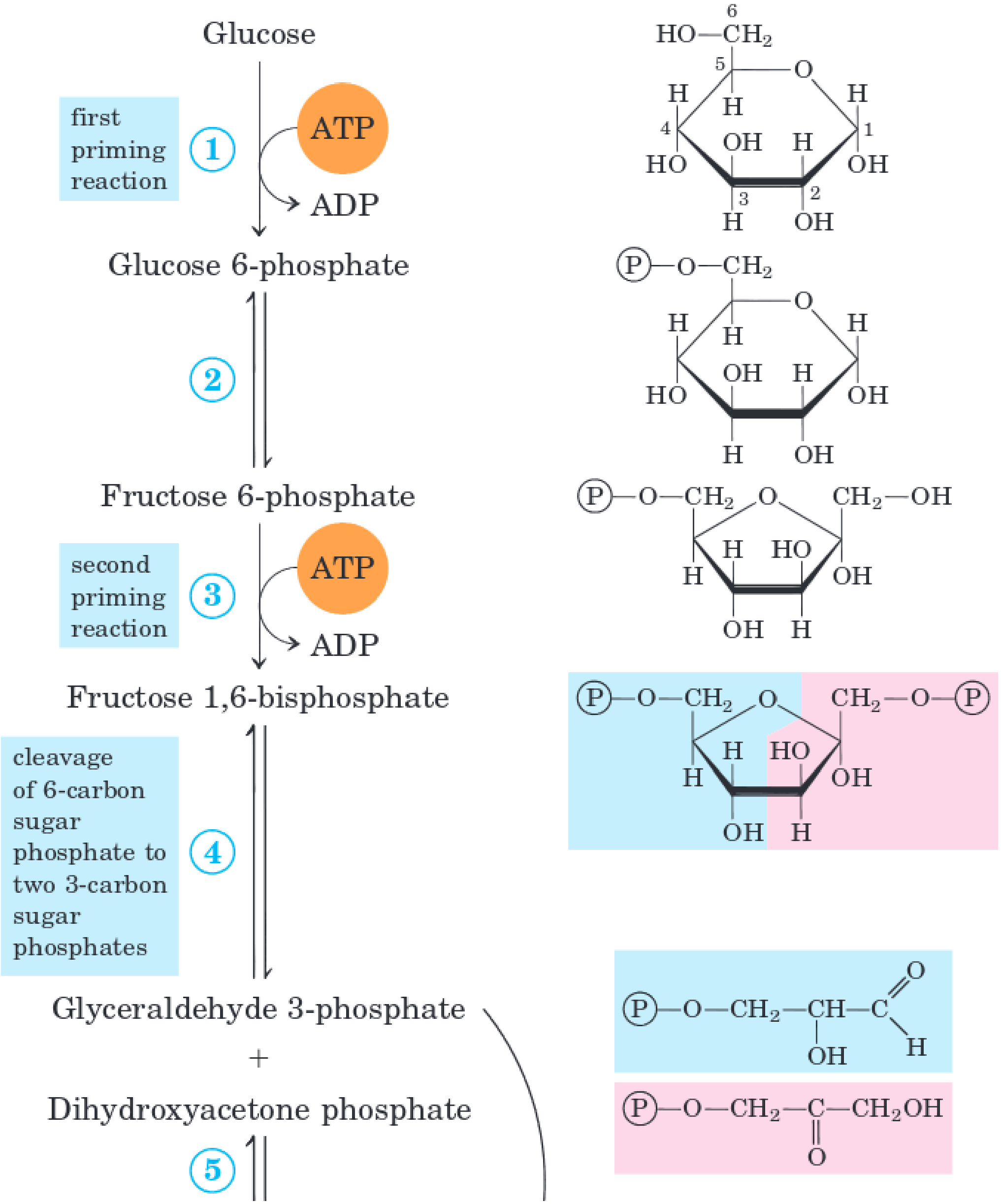
Starch/glycogen

Ribose-5P

GLYCOLYSIS

- Glycolysis (glykys = sweet, lysis= splitting) is a methabolic pathway, where glucose is converted into pyruvate and energy in form of ATP and NADH.
- Very imporant source of energy for many cells.
- Highly conservative: mechanisms, enzyme sequences and structures.
- The best model for many biochemical pathways.
- 10 steps: glucose => pyruvate.
- 2 phases:
  - preparatory phase: ATP energy is invested => glyceraldehyde-3P
  - pay-off phase: ATP, NADH, pyruvate are produced
- Three types of reactions:
  - degradation of glucose
  - ADP => ATP
  - NAD^+ => NADH

GLYCOLYSIS: PREPARATORY PHASE



Glucose

first priming reaction ① ATP → ADP

Glucose 6-phosphate

②

Fructose 6-phosphate

second priming reaction ③ ATP → ADP

Fructose 1,6-bisphosphate

cleavage of 6-carbon sugar phosphate to two 3-carbon sugar phosphates ④

Glyceraldehyde 3-phosphate

+

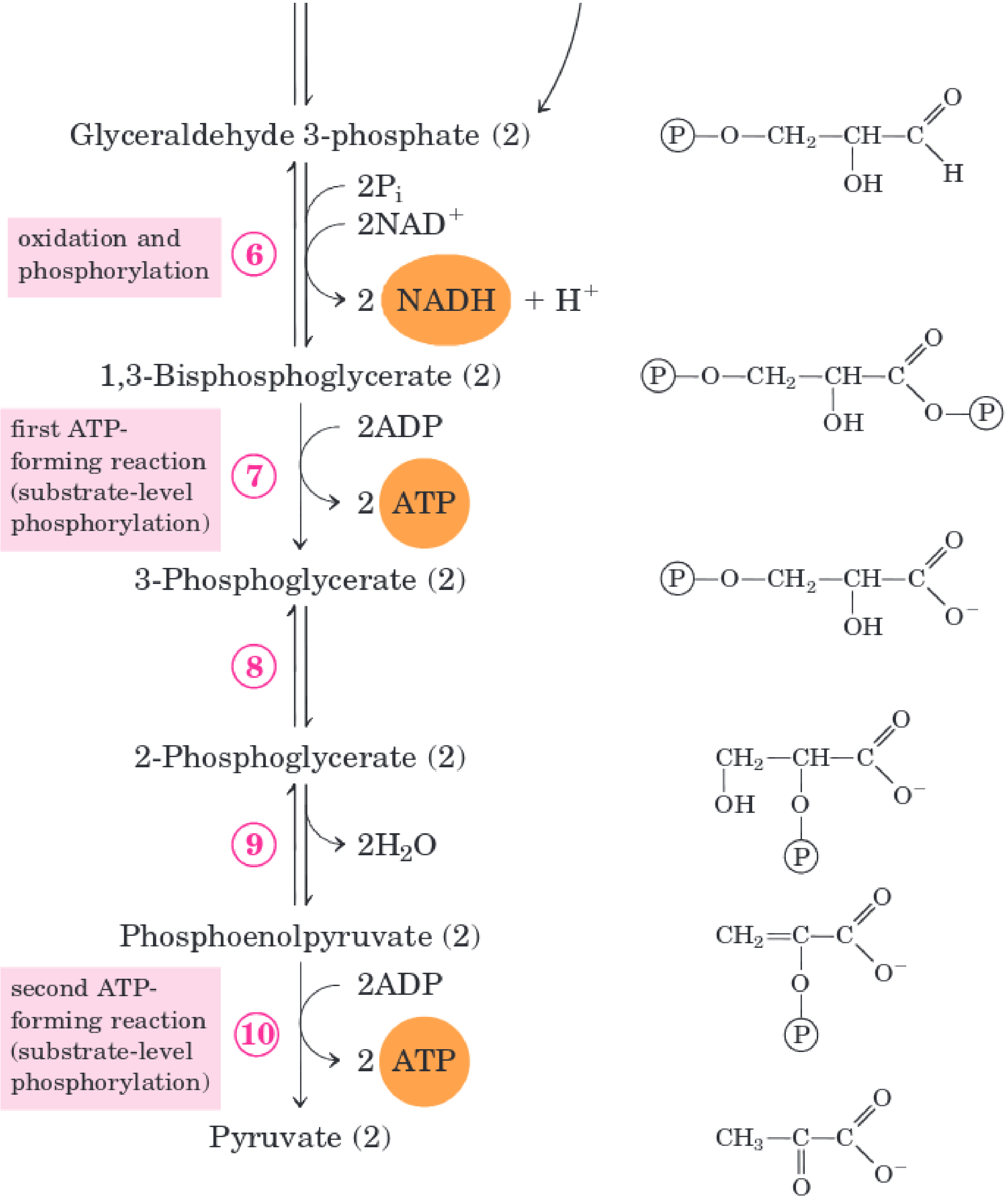
Dihydroxyacetone phosphate

⑤

- ① Hexokinase
- ② Phosphohexose isomerase
- ③ Phospho-fructokinase-1
- ④ Aldolase
- ⑤ Triose phosphate isomerase

Harden and Young experiment (1906): serum + yeast extract => phosphate role in glycolysis

GLYCOLYSIS: PAY-OFF PHASE



Glyceraldehyde 3-phosphate (2)

$2P_i$

$2NAD^+$

$2\ NADH + H^+$

oxidation and phosphorylation ⑥

1,3-Bisphosphoglycerate (2)

2ADP

2 ATP

first ATP-forming reaction (substrate-level phosphorylation) ⑦

3-Phosphoglycerate (2)

⑧

2-Phosphoglycerate (2)

⑨ $2H_2O$

Phosphoenolpyruvate (2)

2ADP

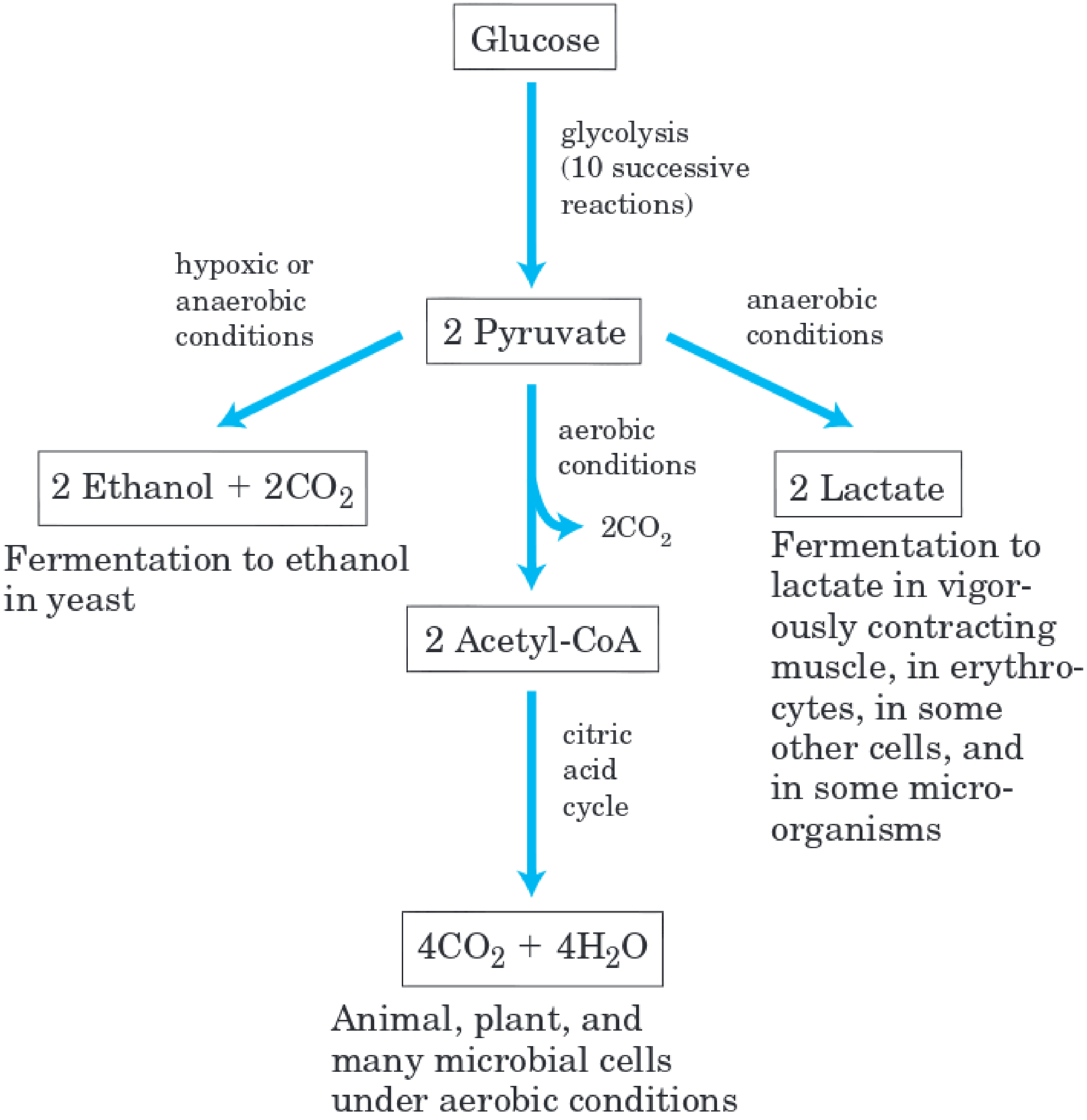
2 ATP

second ATP-forming reaction (substrate-level phosphorylation) ⑩

Pyruvate (2)

- ⑥ Glyceraldehyde 3-phosphate dehydrogenase
- ⑦ Phosphoglycerate kinase
- ⑧ Phosphoglycerate mutase
- ⑨ Enolase
- ⑩ Pyruvate kinase

WHAT HAPPENS WITH PYRUVATE



Glucose

glycolysis
(10 successive
reactions)

2 Pyruvate

hypoxic or
anaerobic
conditions

aerobic
conditions

anaerobic
conditions

$2\ \text{Ethanol} + 2CO_2$

Fermentation to ethanol in yeast

$2CO_2$

2 Acetyl-CoA

citric
acid
cycle

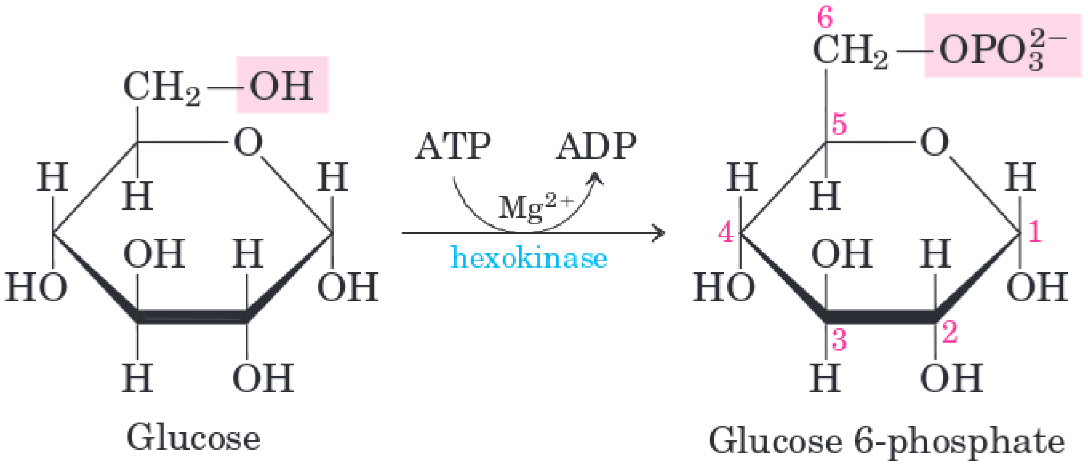
$4CO_2 + 4H_2O$

Animal, plant, and many microbial cells under aerobic conditions

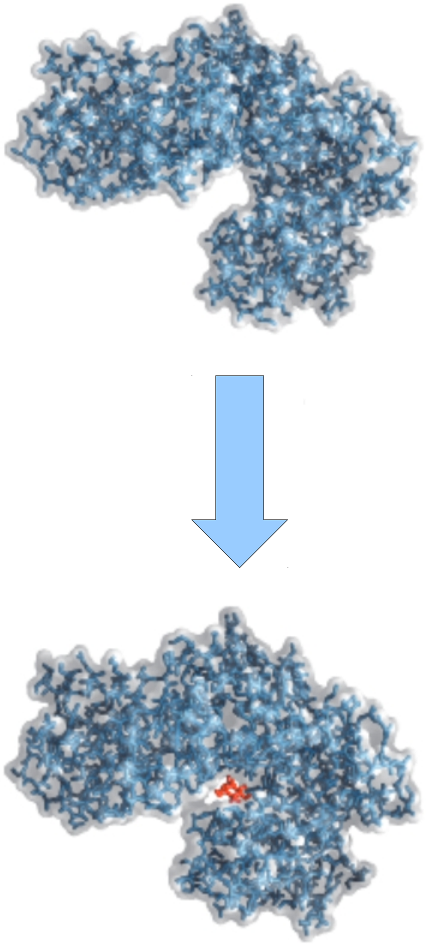
2 Lactate

Fermentation to lactate in vigorously contracting muscle, in erythrocytes, in some other cells, and in some microorganisms

GLYCOLYSIS. STEP 1:
GLUCOSE PHOSPHORYLATION

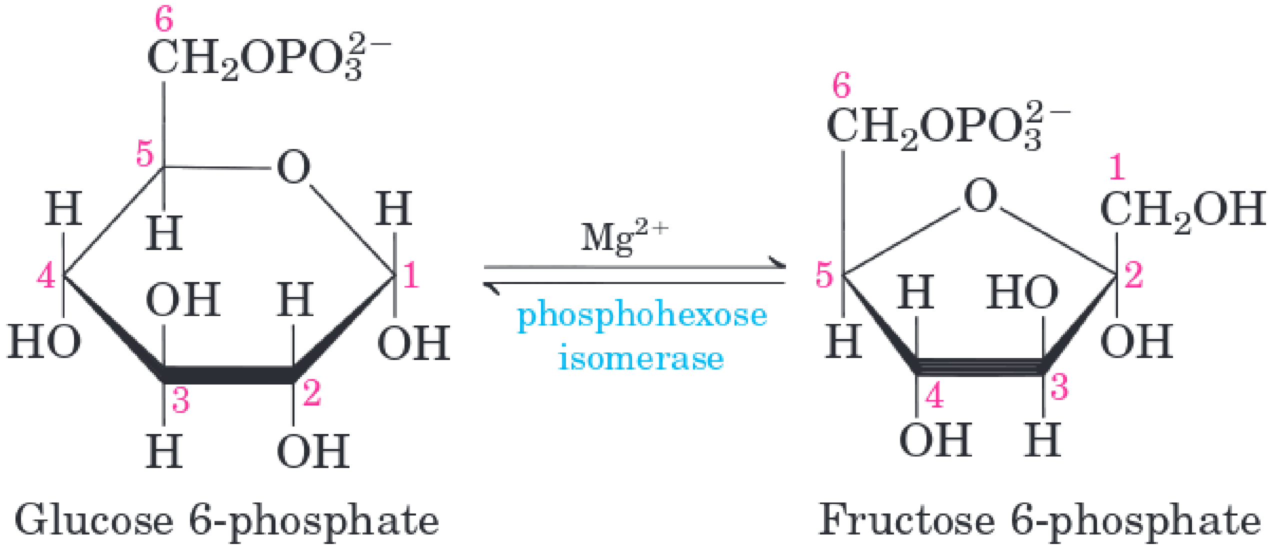


$\Delta G'^{\circ} = -16.7$ kJ/mol



- **Hexokinase: transferase for hexasaccharides, which transfers phosphates.**
- **Cofactors: Mg^{2+}**
- **Conformational changes by binding glucose**
- **Hexokinases are tissue-specific**
- **Exergonic reaction**

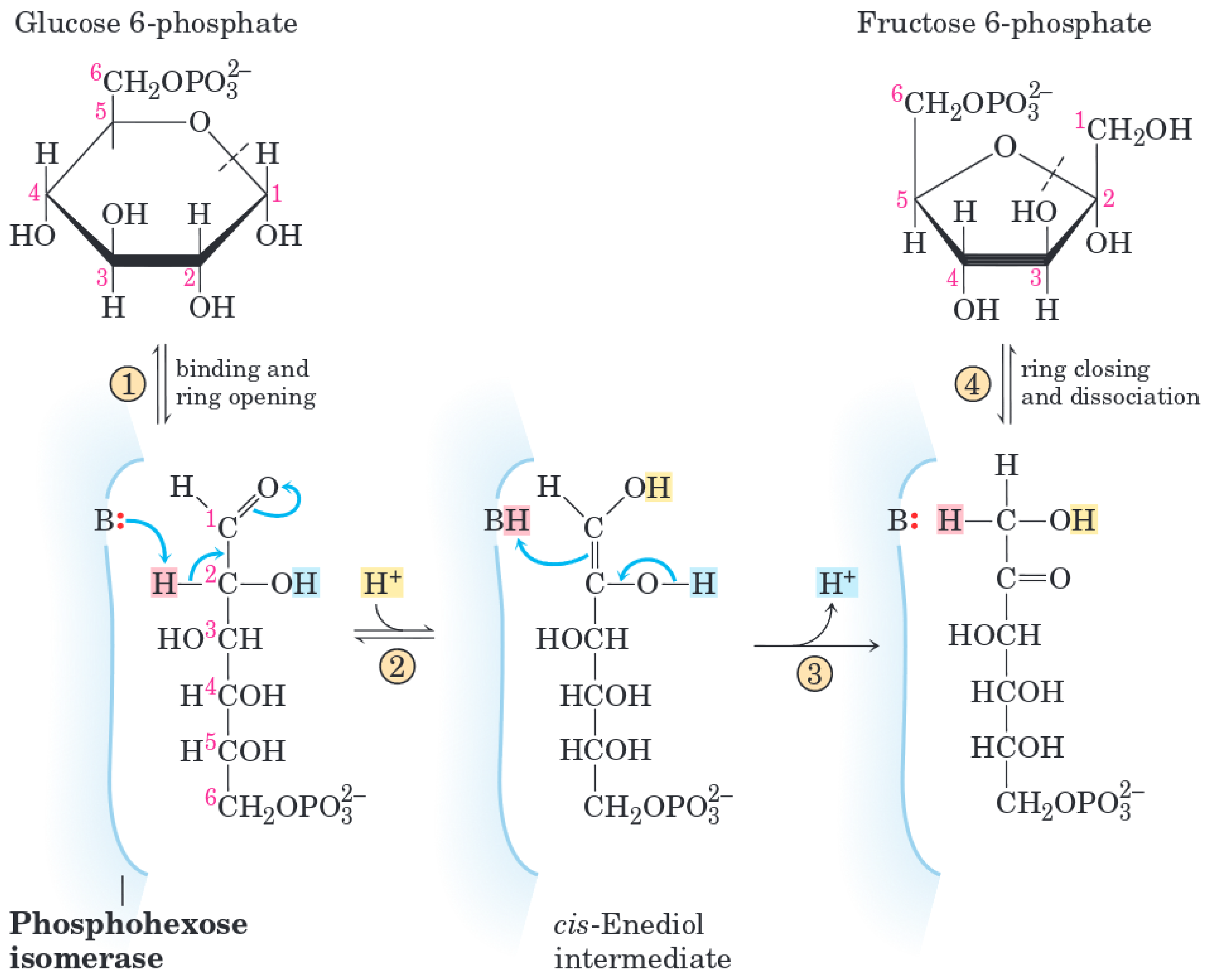
GLYCOLYSIS. STEP 2: CONVERSION OF GLUCOSE-6P TO FRUCTOSE-6P



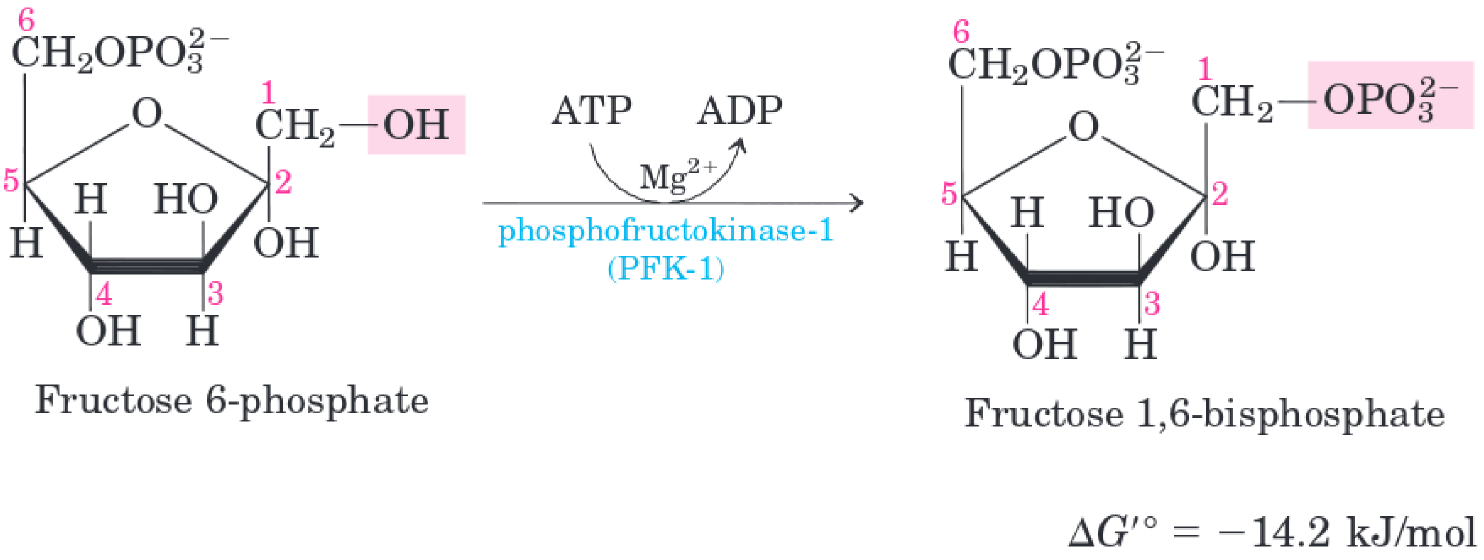
$\Delta G'^{\circ} = 1.7$ kJ/mol

- Phosphohexose isomerase
- Exergonic reaction

ISOMERASE MECHANISM

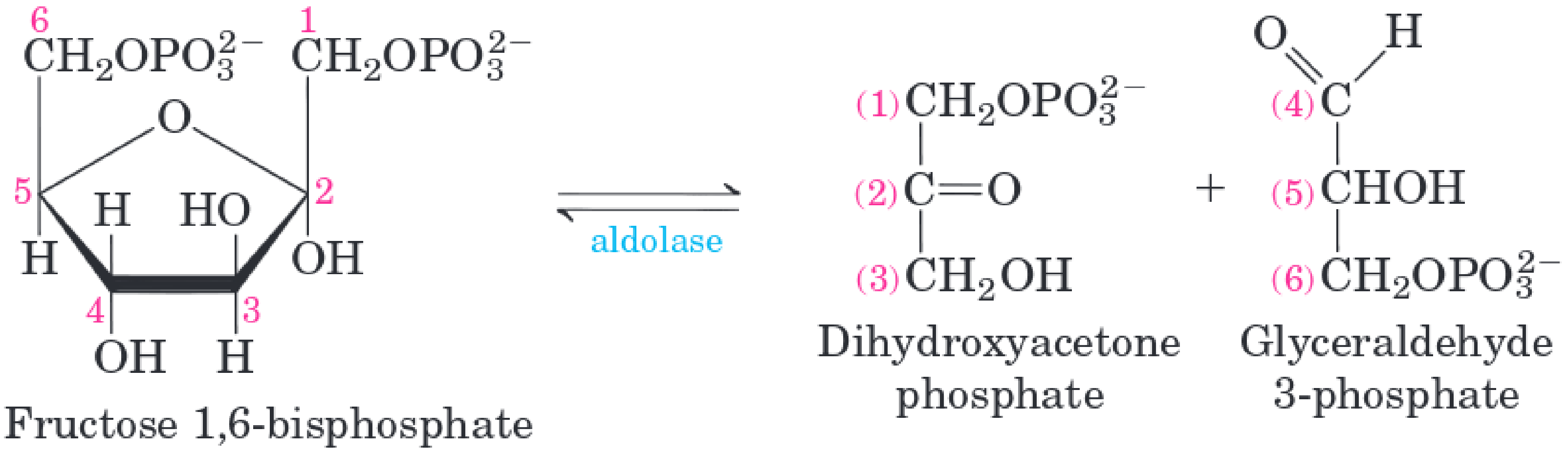


GLYCOLYSIS. STEP 3: PHOSPHORYLATION OF FRUCTOSE-6P TO FRUCTOSE-1,6P



- **Phosphofructokinase-1**
- **PP_i can be used instead of ATP in some plans/bacteria/protists**
- **Regulated: activated by ATP depletion or by ADT/P_i excess; product**
- **Exergonic reaction**

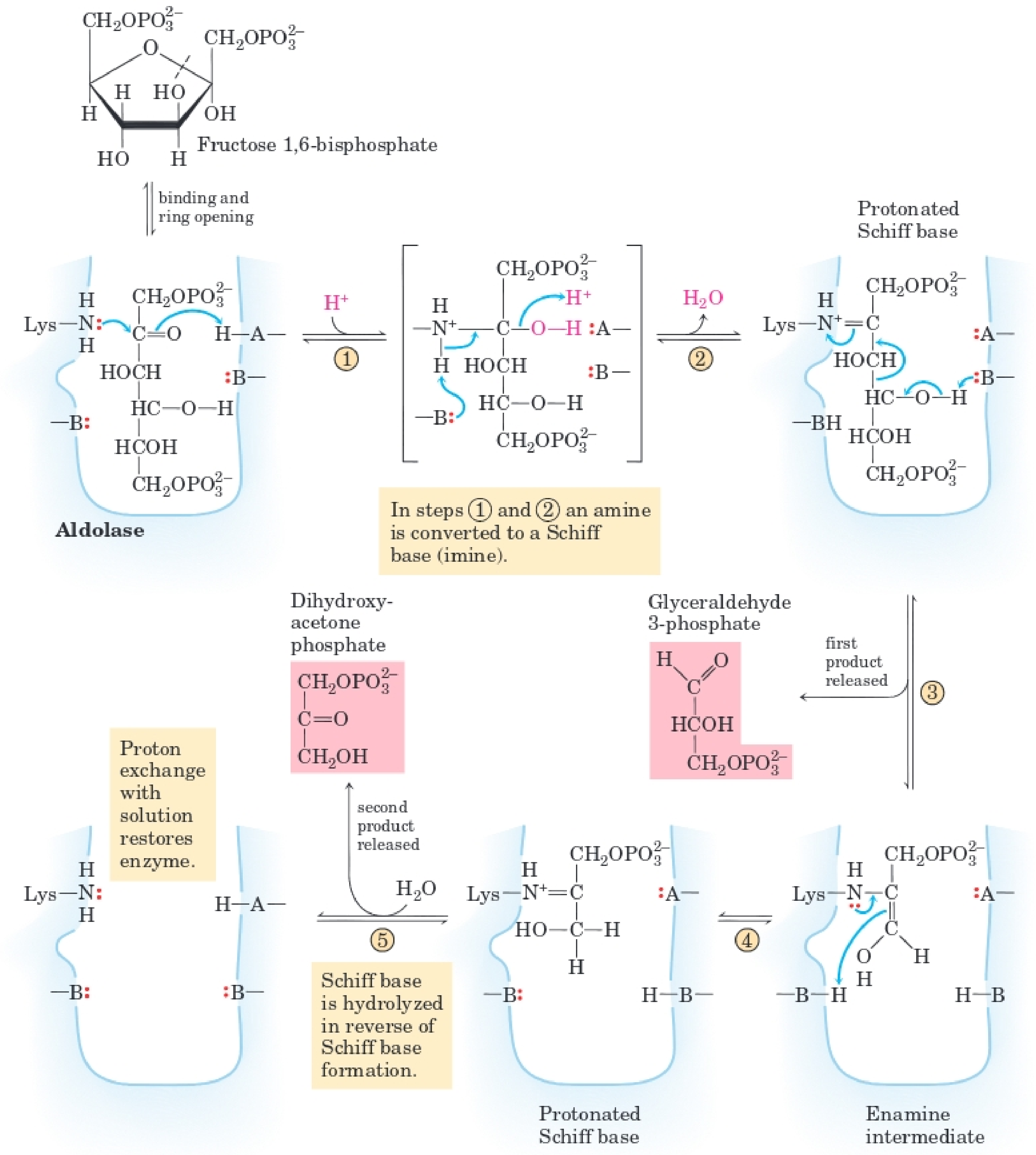
GLYCOLYSIS. STEP 4: CLEAVGE OF FRUCTOSE-1,6P



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

- **Aldolase**
- **Endergenic reaction (could be reversible though at different concentrations)**

ALDOLASE MECHANISM

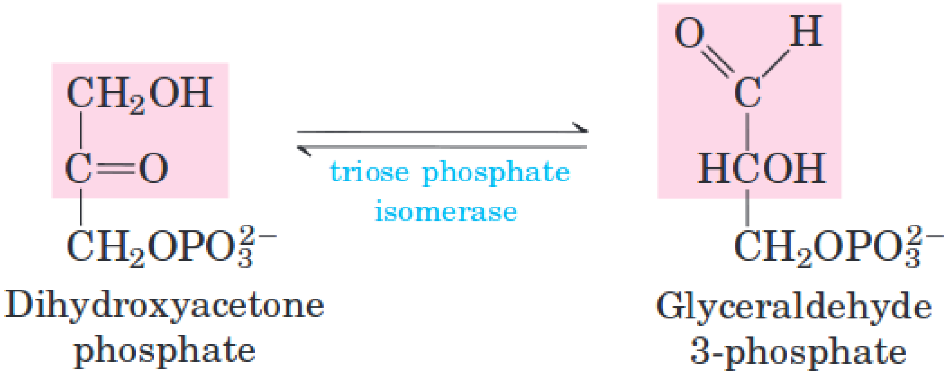


In steps ① and ② an amine is converted to a Schiff base (imine).

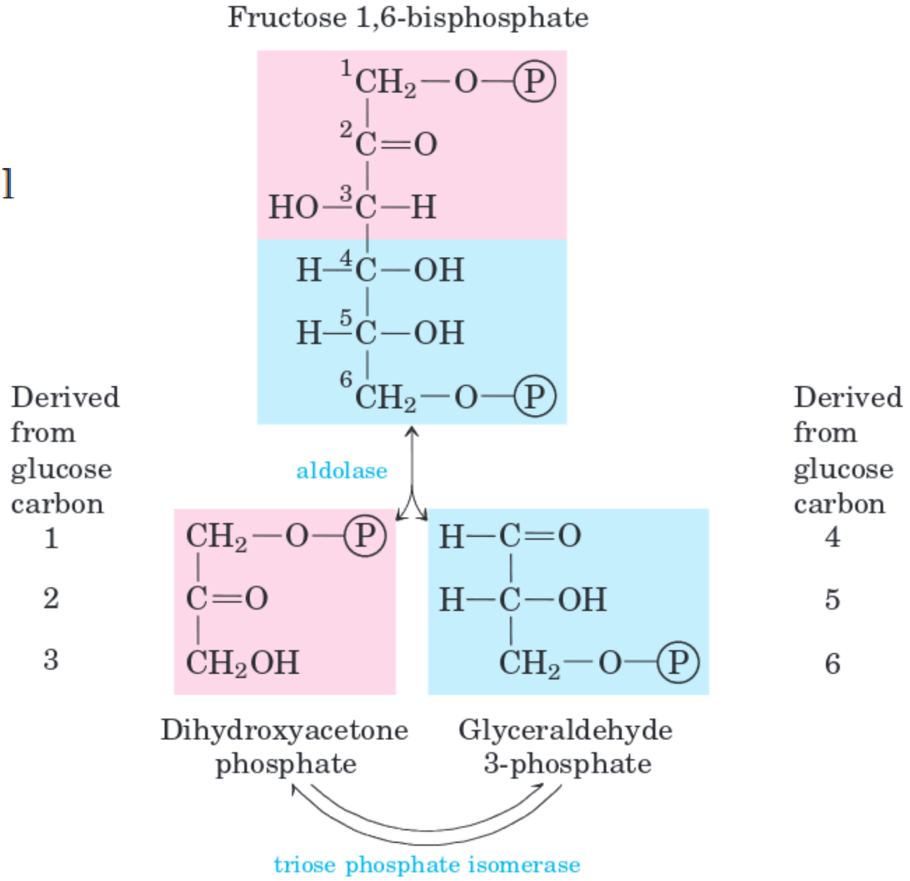
Proton exchange with solution restores enzyme.

Schiff base is hydrolyzed in reverse of Schiff base formation.

GLYCOLYSIS. STEP 5:
INTERCONVERSION OF TRIOSE PHOSPHATES

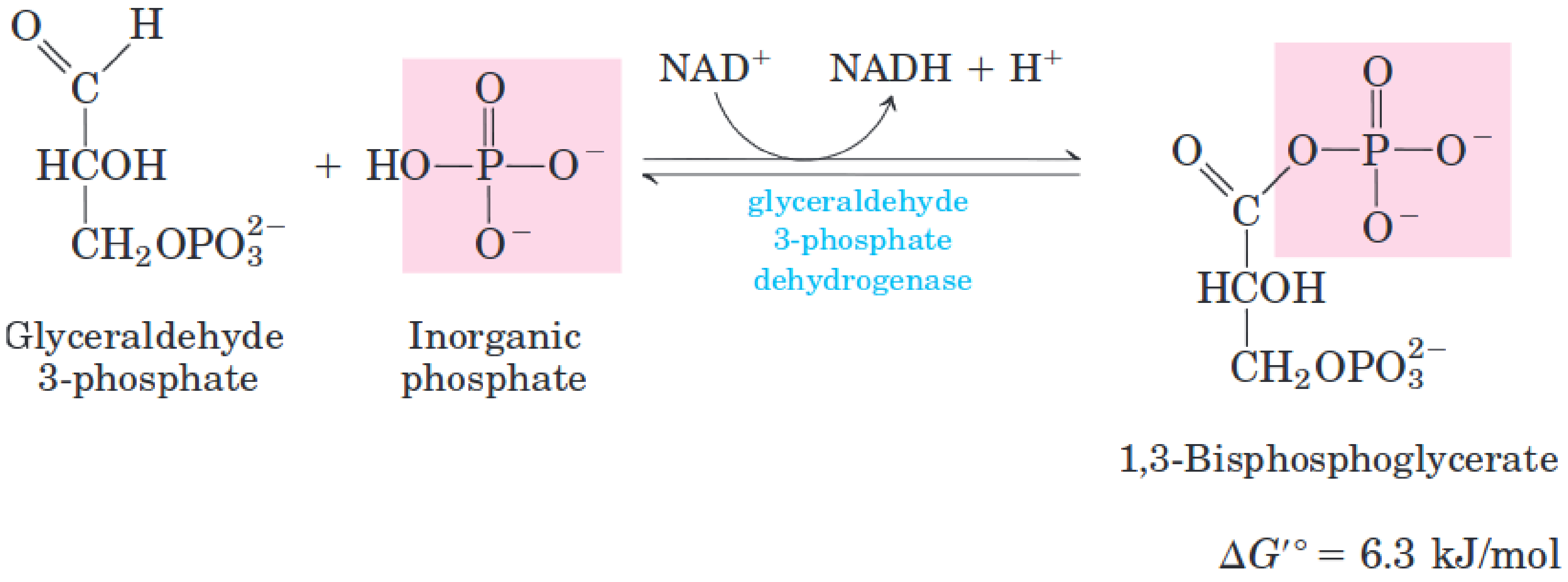


$\Delta G'^{\circ} = 7.5$ kJ/mol



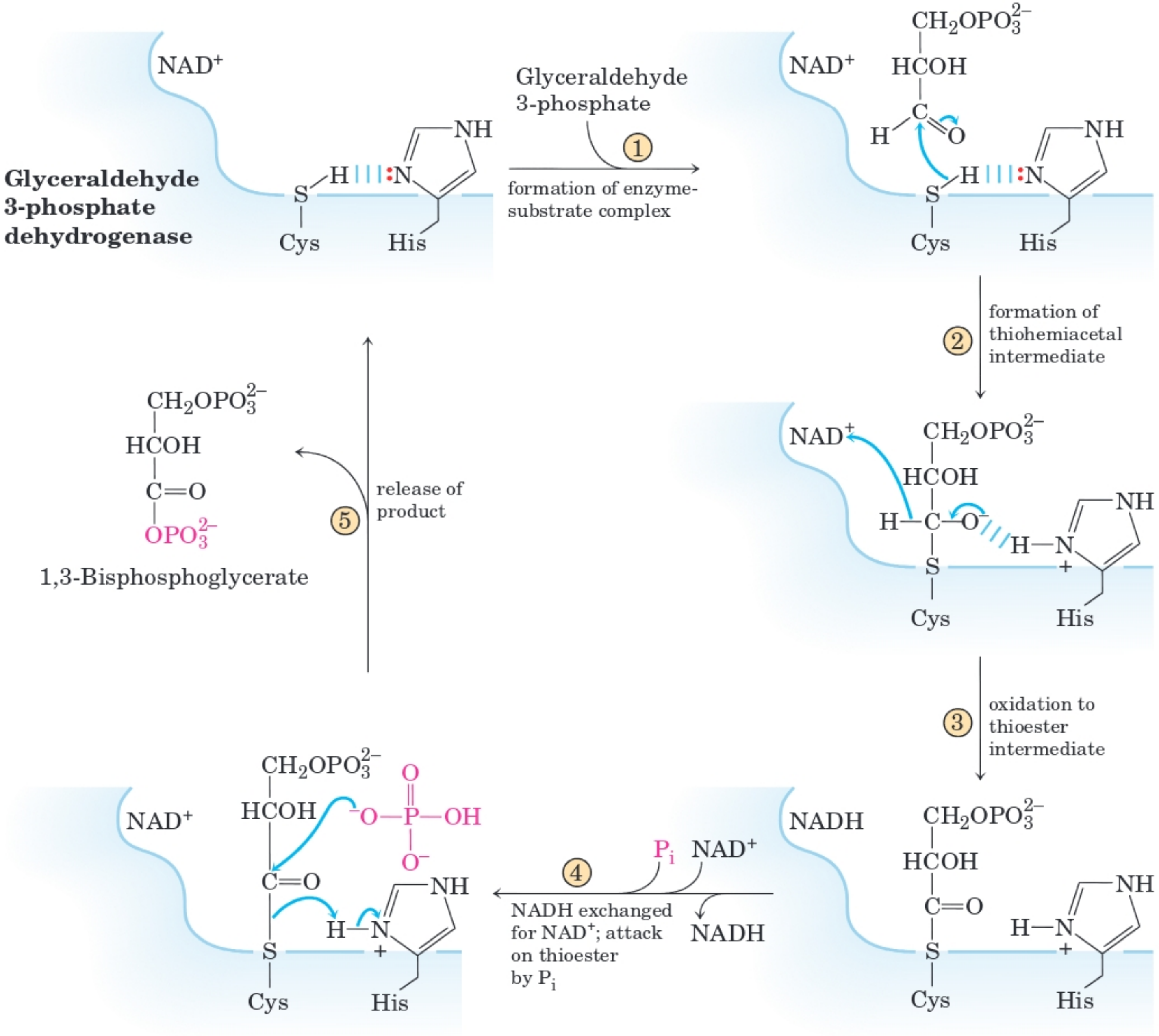
- **Triose phosphate isomerase**
- **Endergonic reaction**

GLYCOLYSIS. STEP 6:
OXIDATION OF GLYCERALDEHYDE 3-P TO 1,3-BIPHOSPHOGLYCERATE

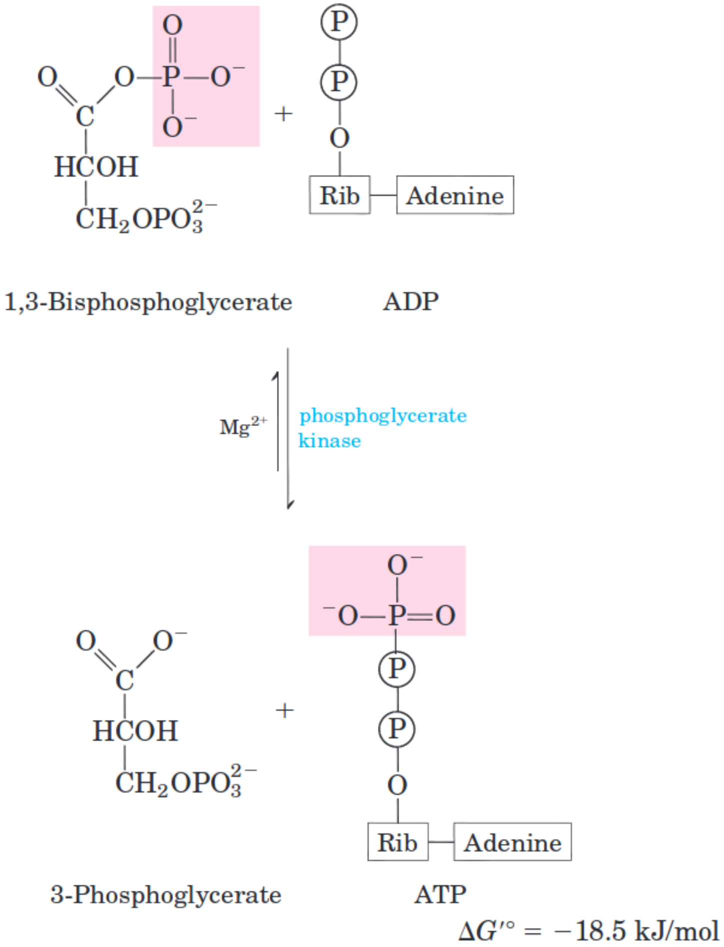


- **Glyceraldehyde 3-phosphate dehydrogenase**
- **NAD^+-limited => process stops when $[NAD^+]$ drops**
- **Endergonic reaction**

GLYCERALDEHYDE 3-PHOSPHATE DEHYDROGENASE MECHANISM

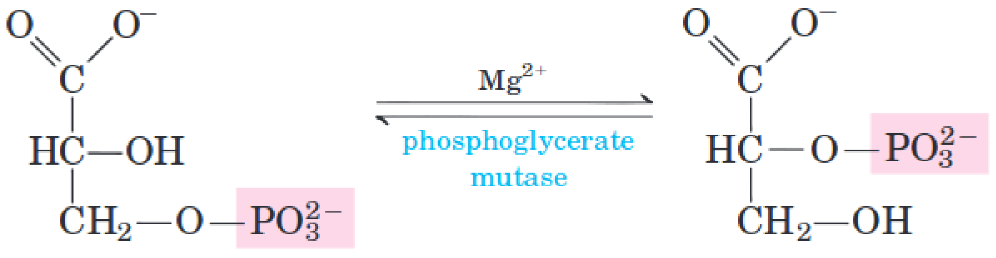


GLYCOLYSIS. STEP 7: PHOSPHORYL TRANSFER FROM 1,3-BIPHOSPHOGLYCERATE TO ADP



- Phosphoglycerate kinase
- Coupled with the step 6: substrate-level phosphorylation
- Exergonic reaction

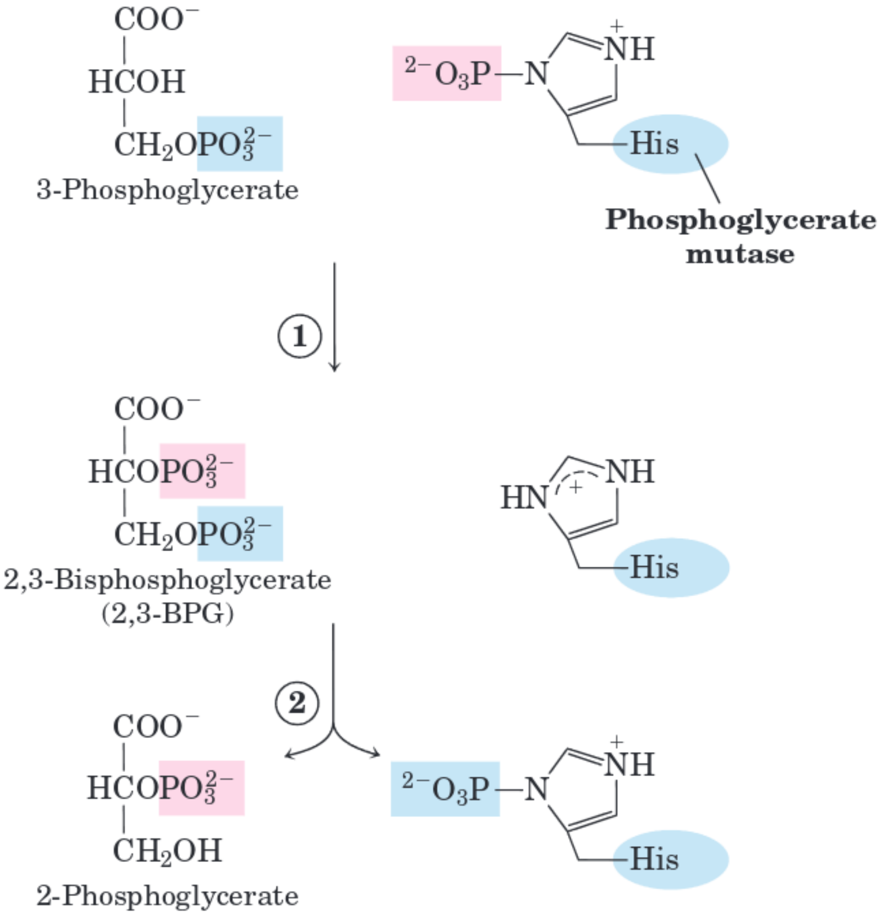
GLYCOLYSIS. STEP 8: CONVERSION OF 3-PHOSPHOGLYCERATE TO 2-PHOSPHOGLYCERATE



3-Phosphoglycerate

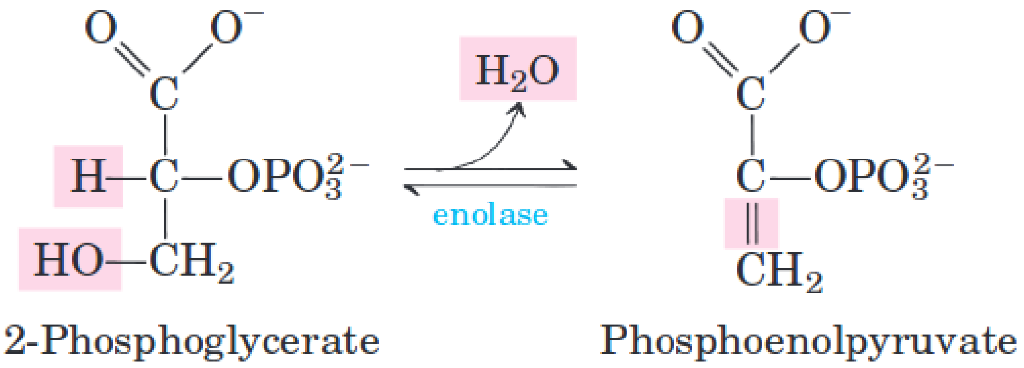
2-Phosphoglycerate

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



- **Phosphoglycerate mutase**
- **Endergonic reaction**

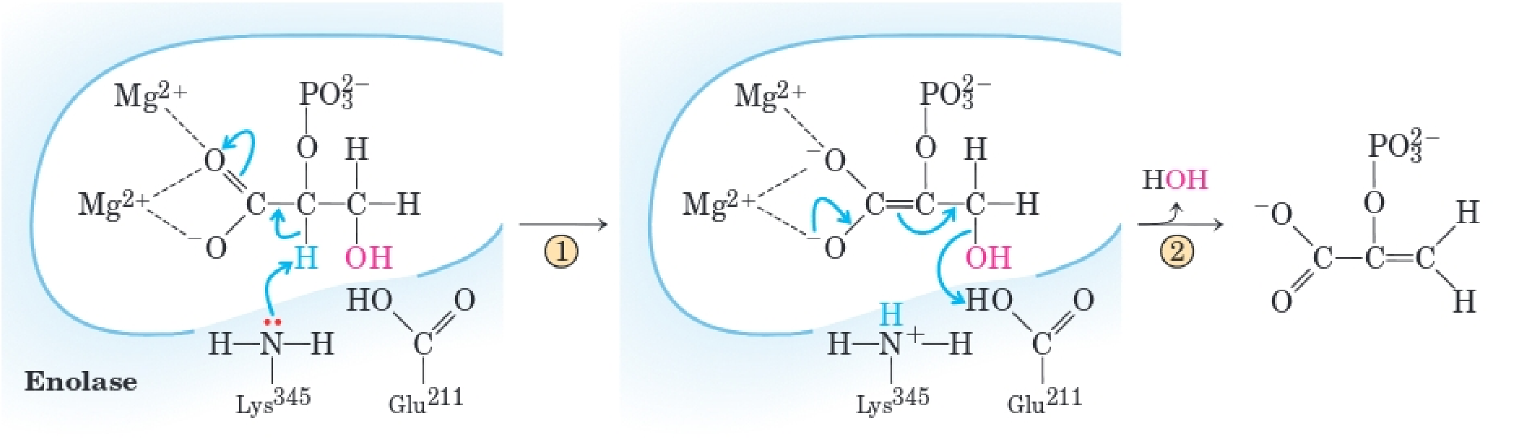
GLYCOLYSIS. STEP 9: DEHYDRATION OF 2-PHOSPHOGLYCERATE TO PHOSPHOENOLPYRUVATE



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

- **Enolase**
- **Cofactors: Mg^{2+}**
- **Endergonic reaction**

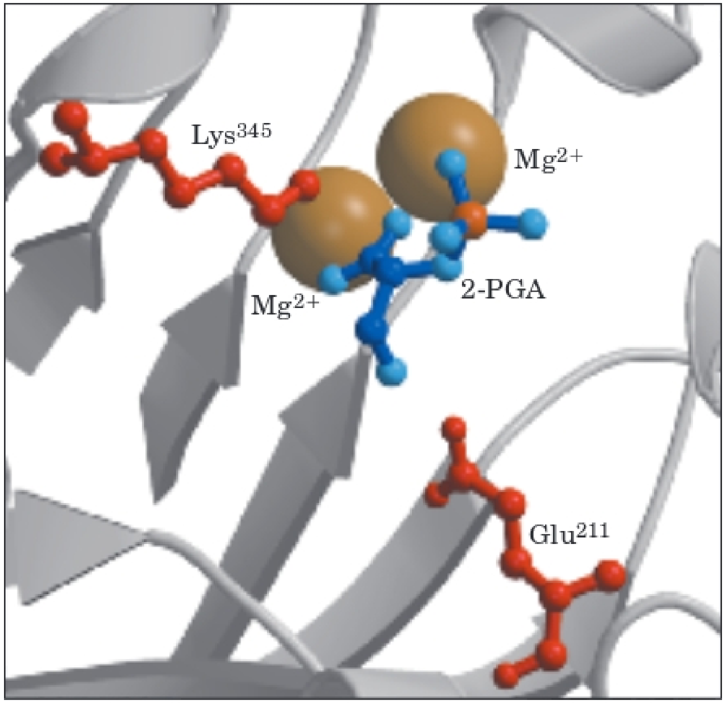
ENOLASE MECHANISM



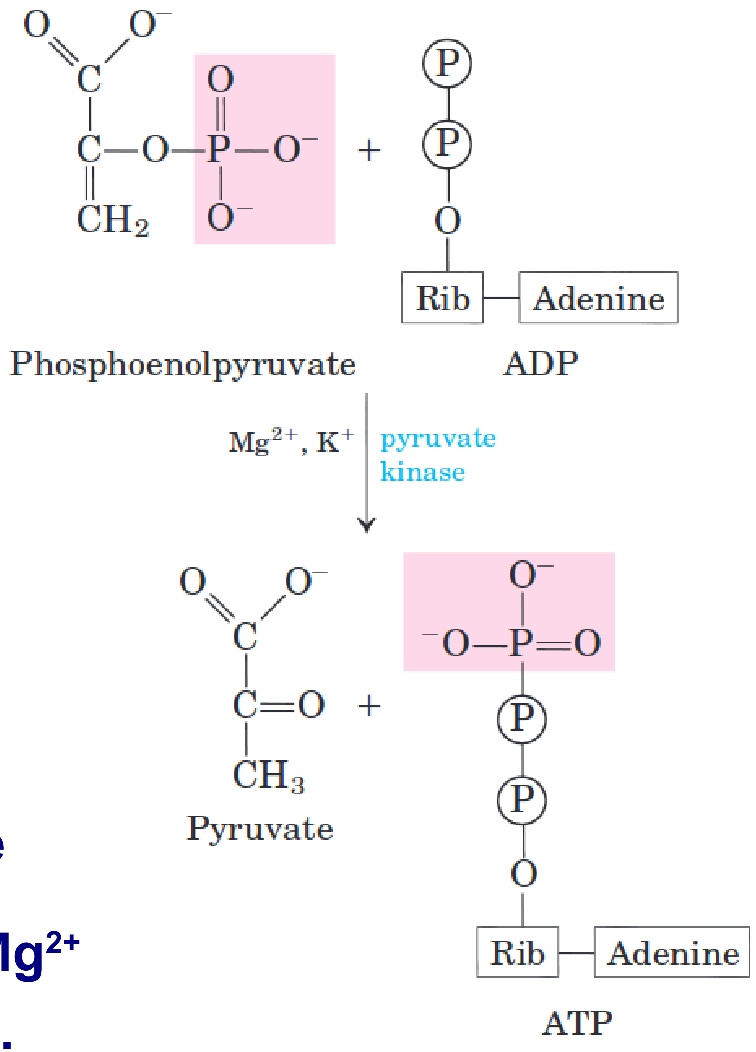
2-Phosphoglycerate bound to enzyme

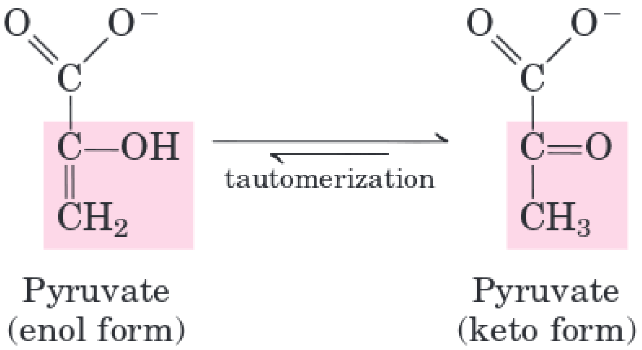
Enolic intermediate

Phosphoenolpyruvate



GLYCOLYSIS. STEP 10: TRANSFER OF THE PHOSPHORYL GROUP FROM PHOSPHOENOLPYRUVATE TO ADP





- **Pyruvate kinase**
- **Cofactors: K^+, Mg^{2+}**
- **Exergonic reaction**

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

GLYCOLYSIS: REACTION BALANCE

- **Overal balance:**

  Glucose + $2NAD^+$ + 2ADP + $2P_i$ => 2pyruvate + NADH + $2H^+$ + 2ATP +$2H_2O$

- **Glucose convertion:**

  Glucose + $2NAD^+$ => 2pyruvate + NADH + $2H^+$; $\Delta G'^0 = -146$ kJ/mol

  NADH + $2H^+$ + O_2 => NAD^+ + $2H_2O$ (=> respirational phosphorylation)

- **ATP formation:**

  2ADP + $2P_i$ => 2ATP + $2H_2O$; $\Delta G'^0 = 61.0$ kJ/mol

- **Total $\Delta G'^0 = -85$ kJ/mol**

Role of phosphorylated intermediates:

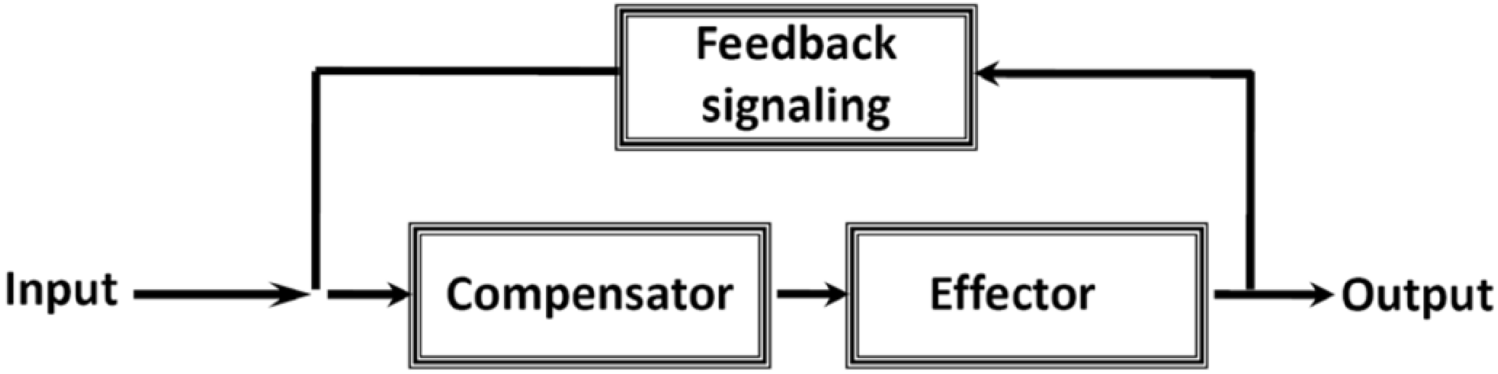
- Signal to remain in the cell independently of the concentrations

- Donors of P_i to ADP => ATP

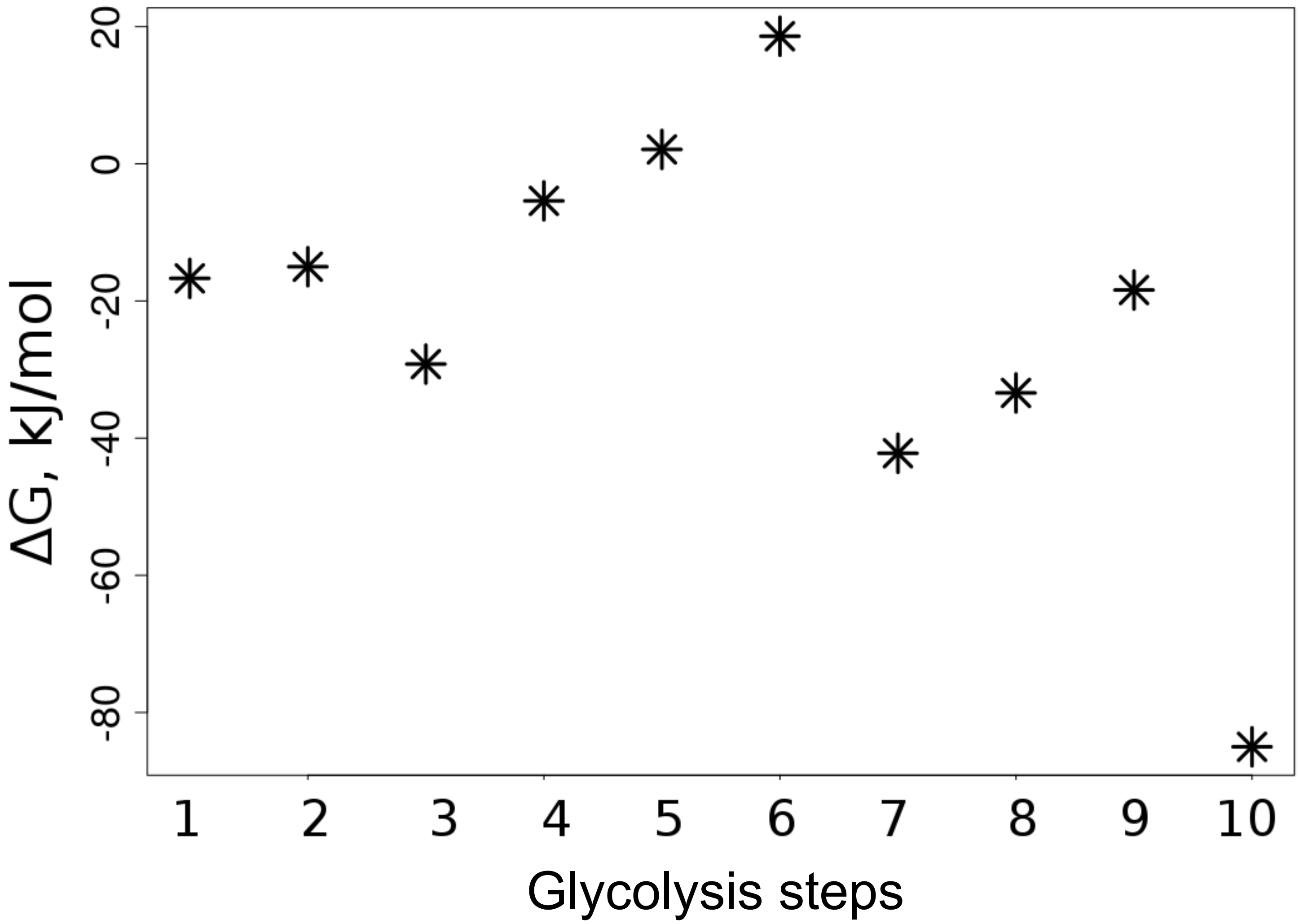
- Allosteric regulation of enzymes

GLYCOLYSIS REGULATION

- Aerobic/anaeronic conditions (~ 1 order more effective in anaerobic)
- ATP consumption (ATP/ADP balance)
- NADH regeneration (NAD^+/NADH balance)
- Allosteric regulation of the enzymes (hexokinase, PFK-1, pyruvate kinase)
- Feedback from the metabolites
- Hormones: glucagon, epinephrine, insulin
- Expression level of all the enzymes



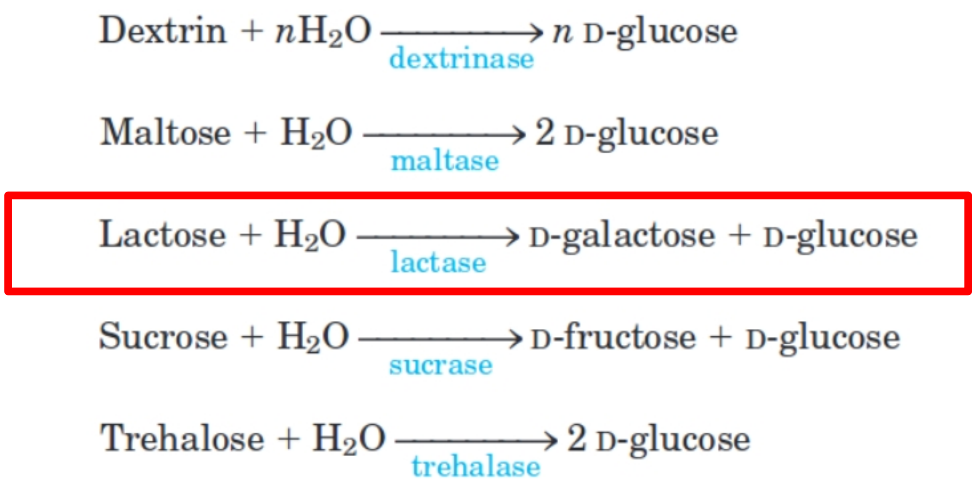
GLYCOLYSIS: ENERGY DIAGRAM



GLYCOLYSIS: FEEDER PATHWAYS

Glucose can be obtained from different sources to enter glycolysis:

- **Glycogen and starch:**
  - phosphorylation
  - debranching
  - mutase catalyzed reaction (Glu-1P => Glu-6P)
  - isomerization
- **Deitary polysaccharides**
  - hydrolyzation (α-amylase)
  - hydrolyzation of dimers



$$\text{Dextrin} + n\text{H}_2\text{O} \xrightarrow[\text{dextrinase}]{} n\ \text{D-glucose}$$

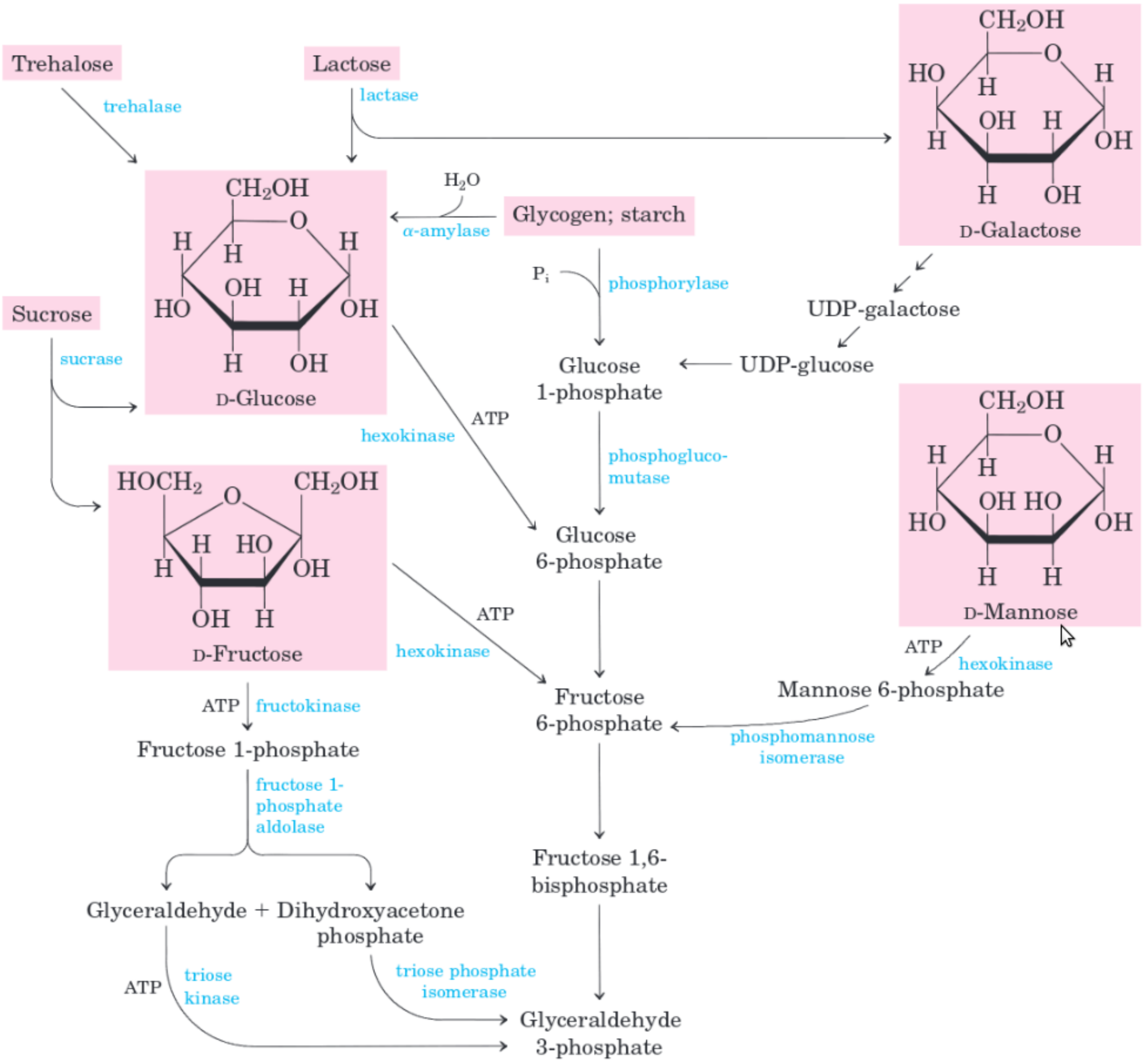
$$\text{Maltose} + \text{H}_2\text{O} \xrightarrow[\text{maltase}]{} 2\ \text{D-glucose}$$

$$\text{Lactose} + \text{H}_2\text{O} \xrightarrow[\text{lactase}]{} \text{D-galactose} + \text{D-glucose}$$

$$\text{Sucrose} + \text{H}_2\text{O} \xrightarrow[\text{sucrase}]{} \text{D-fructose} + \text{D-glucose}$$

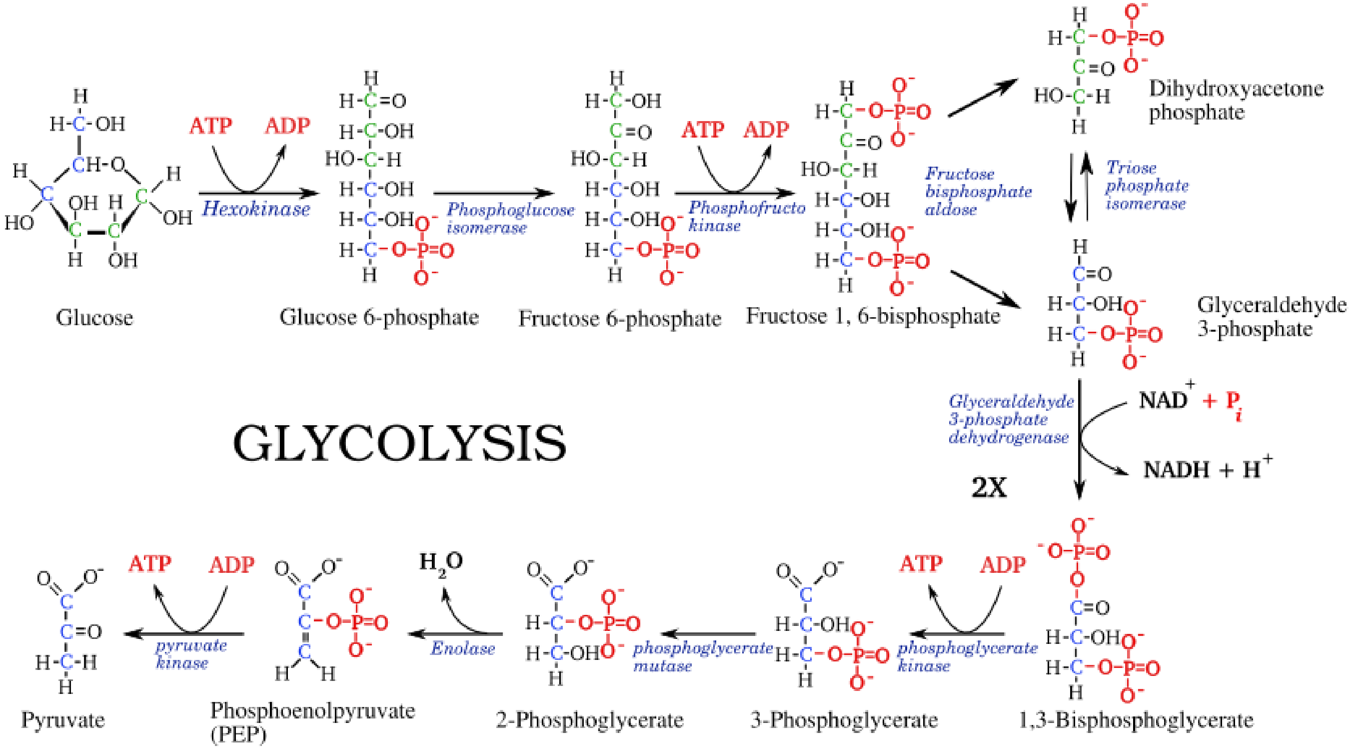
$$\text{Trehalose} + \text{H}_2\text{O} \xrightarrow[\text{trehalase}]{} 2\ \text{D-glucose}$$

GLYCOLYSIS: FEEDER PATHWAYS



GLYCOLISIS: SUMMARY

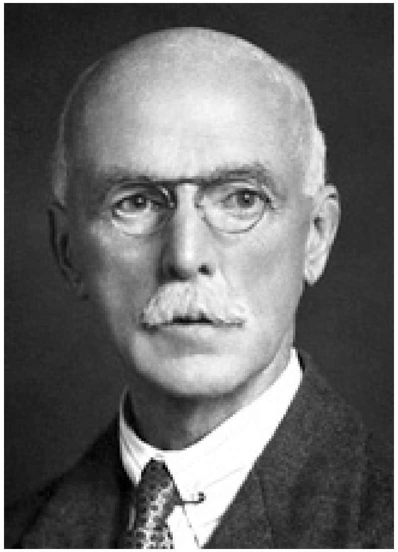
- **Universal pathway for degrading glucose to two pyruvates with the production of energy in form of ATP and NADH.**
- **All the enzymes are cytoplasmic.**
- **All the intermediates have either hexa- or tricarbonic chains.**
- **Two phases: preparatory and pay-off.**



FERMENTATION

Arthur Harden (1865-1940):

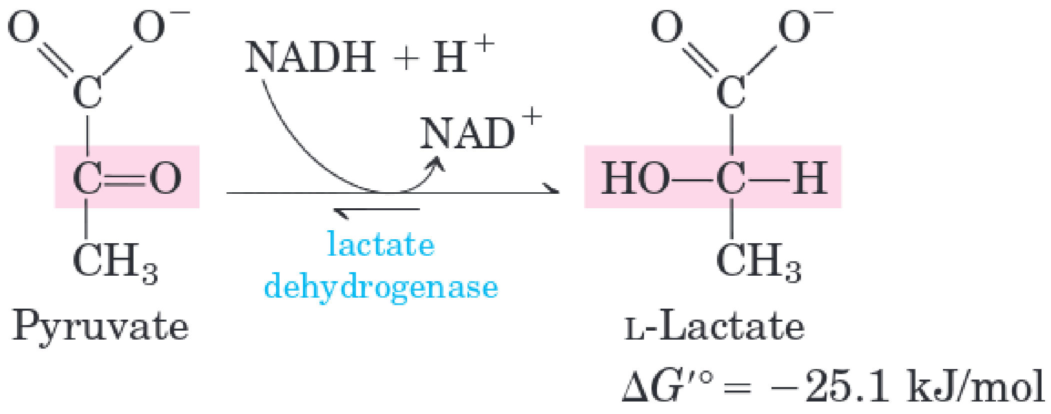
"The problem of alcoholic fermentation, of the origin and nature of that mysterious and apparently spontaneous change, which converted the insipid juice of the grape into stimulating wine, seems to have exerted a fascination over the minds of natural philosophers from the very earliest times."

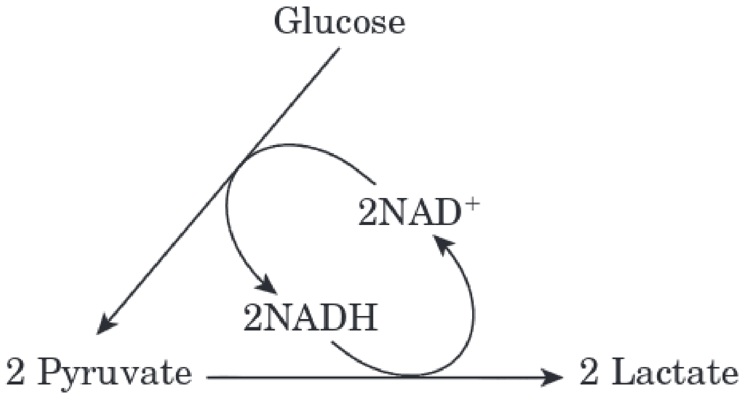


FERMENTATION

Fermentation: anaerobic degradation of pyruvate to ethanol or lactate.

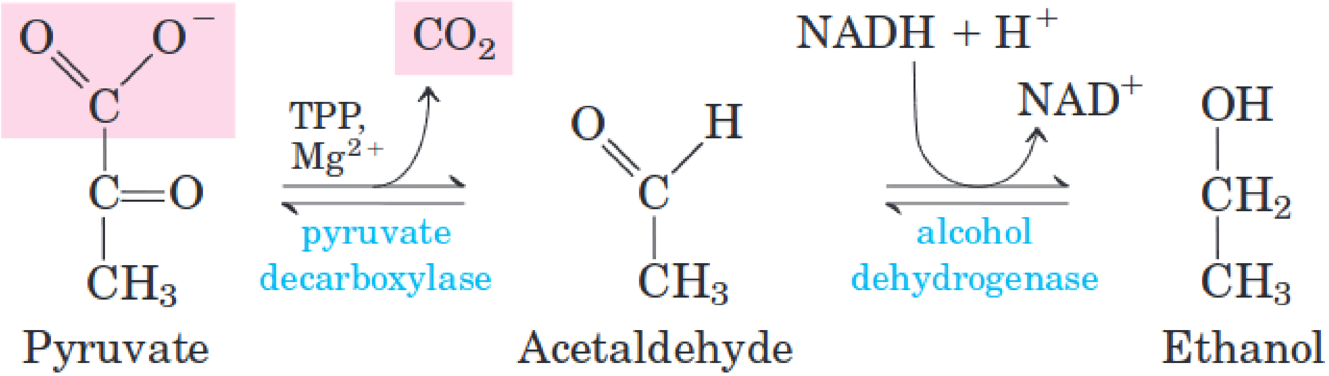
In this process, the energy is extracted but O_2 is not consumed and NAD+/NADH balance is not changed.



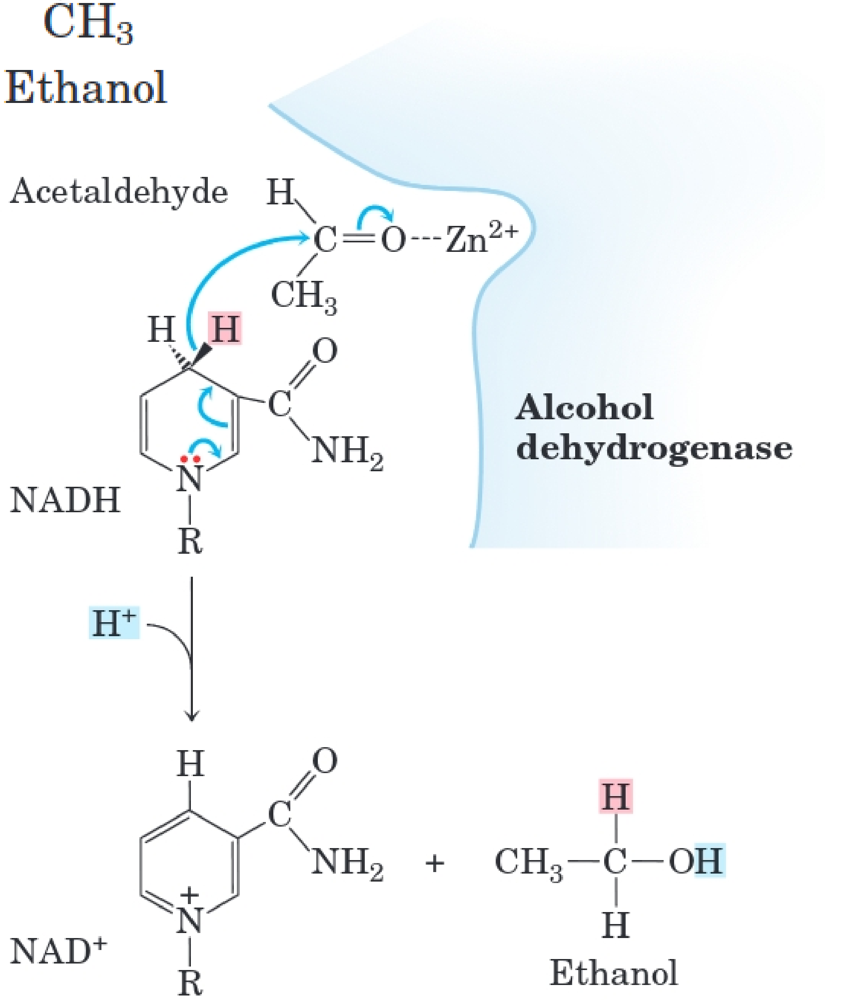


- **Lactate dehydrogenase**
- **Muscles**
- **Conditions: not enough O_2 and NAD+**
- **Exergonic reaction**

FERMENTATION



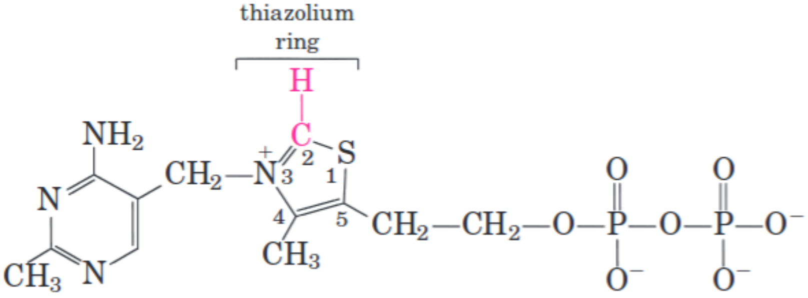
- **Pyruvate decarboxylase and alcohol dehydrogenase**
- **Cofactor: Mg^{2+}, thiamine pyrophosphate**
- **Yeasts and microorganisms**



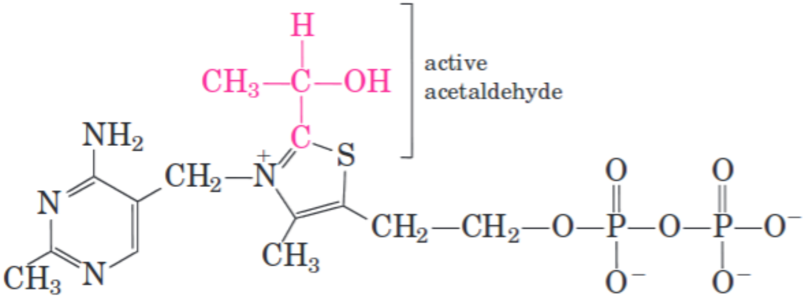
Glucose + 2ADP + 2Pi =>

2ethanol + $2CO_2$ + 2ATP +$2H_2O$

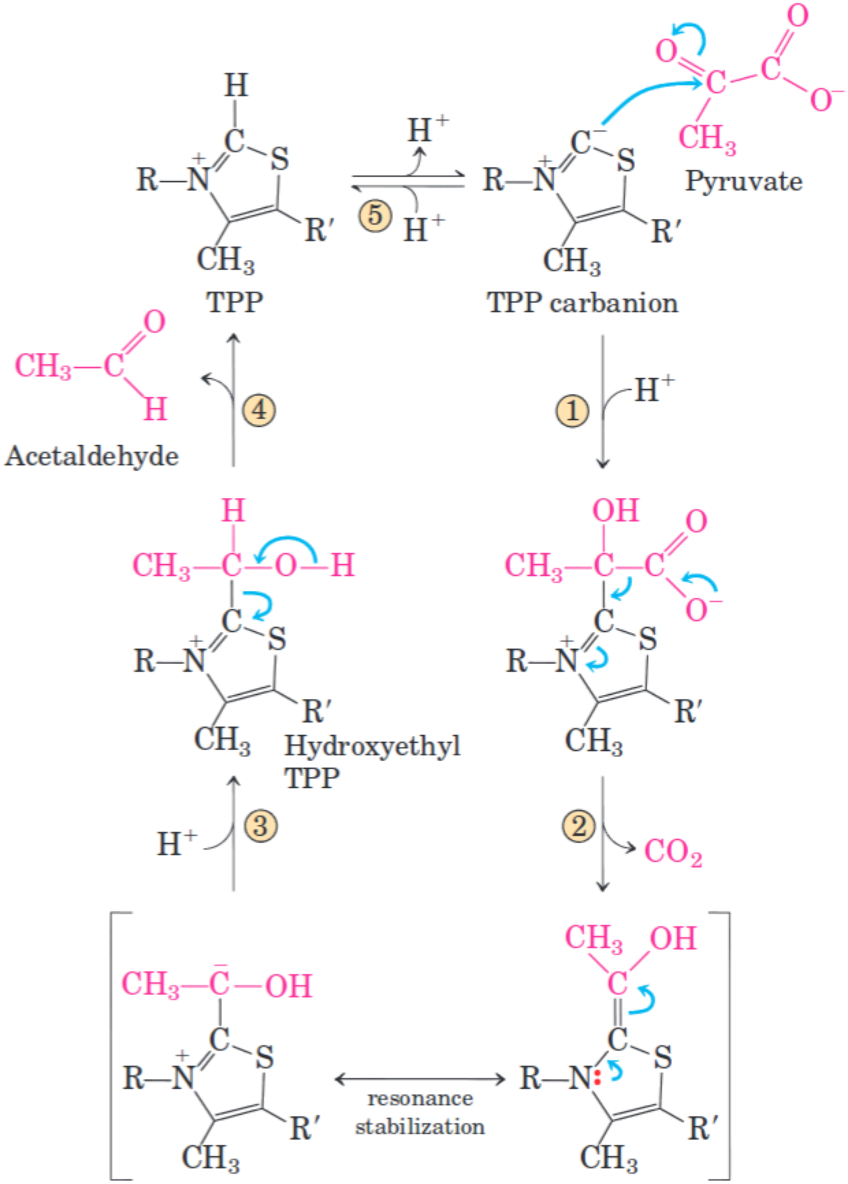
THIAMINE PYROPHOSPHATE (TPP)



Thiamine pyrophosphate (TPP)



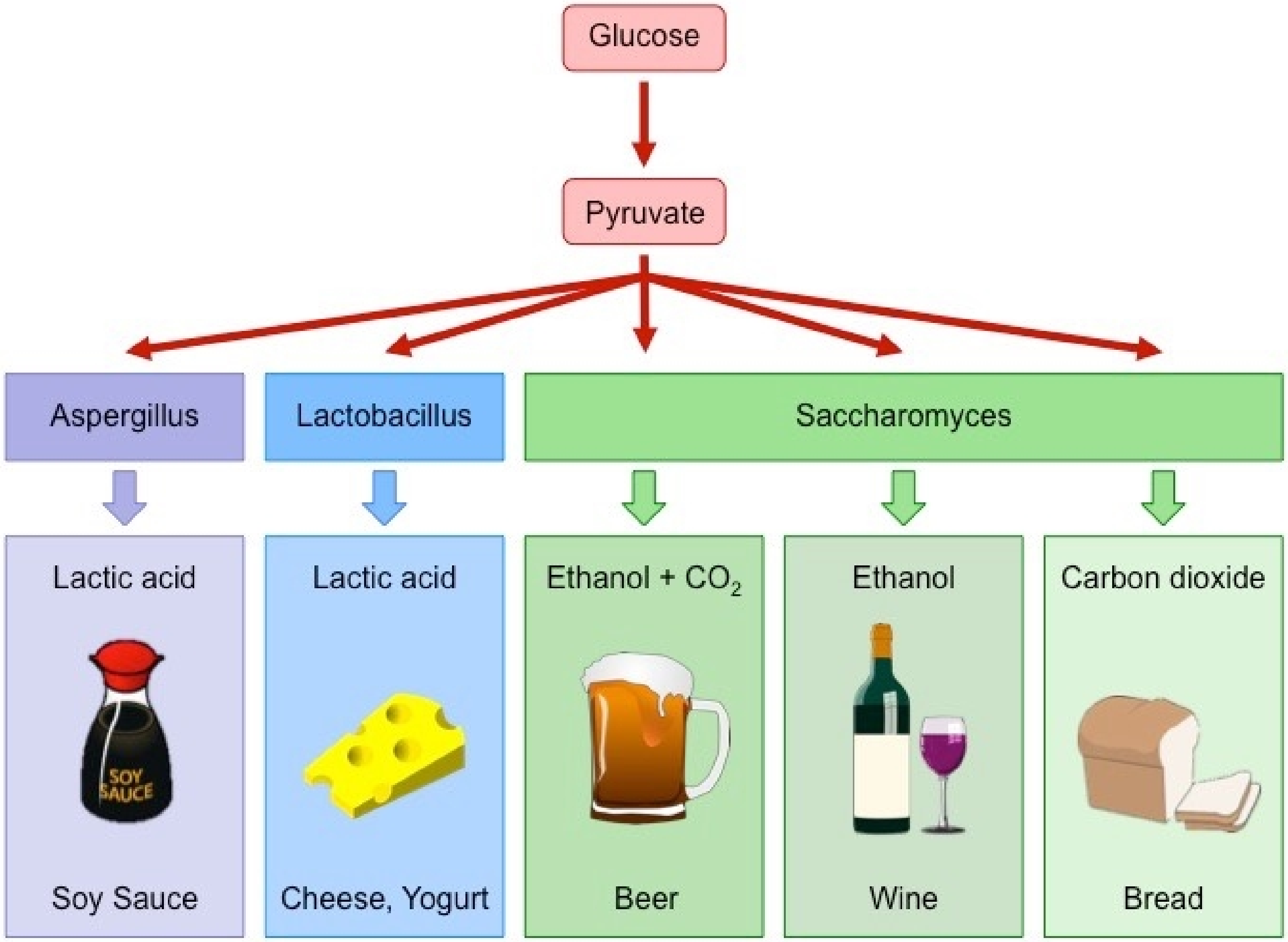
Hydroxyethyl thiamine pyrophosphate



Derivative of B1 vitamin

PRODUCTS OF FERMENTATION

- **Yogurt**
- **Cheese**
- **Pickles**
- **Sauerkraut**
- **Soy sauce**
- **Alcohols**



BREWING BEER

Carbohydrates (barley)

Germination (enzymes)
+ water; - cell rests

Oligosaccharides (malt)

Air + yeast cells:
- glycolysis (O_2 is avalable)
- fermentation

Raw beer

Proteolitic enzymes
Filtering

Beer

LECTURE 3-4: INTRODUCTION TO CELL CHEMISTRY AND BIOSYNTHESIS II

- Principles of bioenergetics:
  - metabolism, anabolism, catabolism
  - metabolic reaction types
  - thermodynamics
- ATP hydrolysis
- Carriers in molecular biosythesis: ATP, NAD(P)H, FAD
- Oxidation-reduction reactions
- Examples of metabolic pathways:
  - photosynthesis
  - glycolysis
  - fermentation