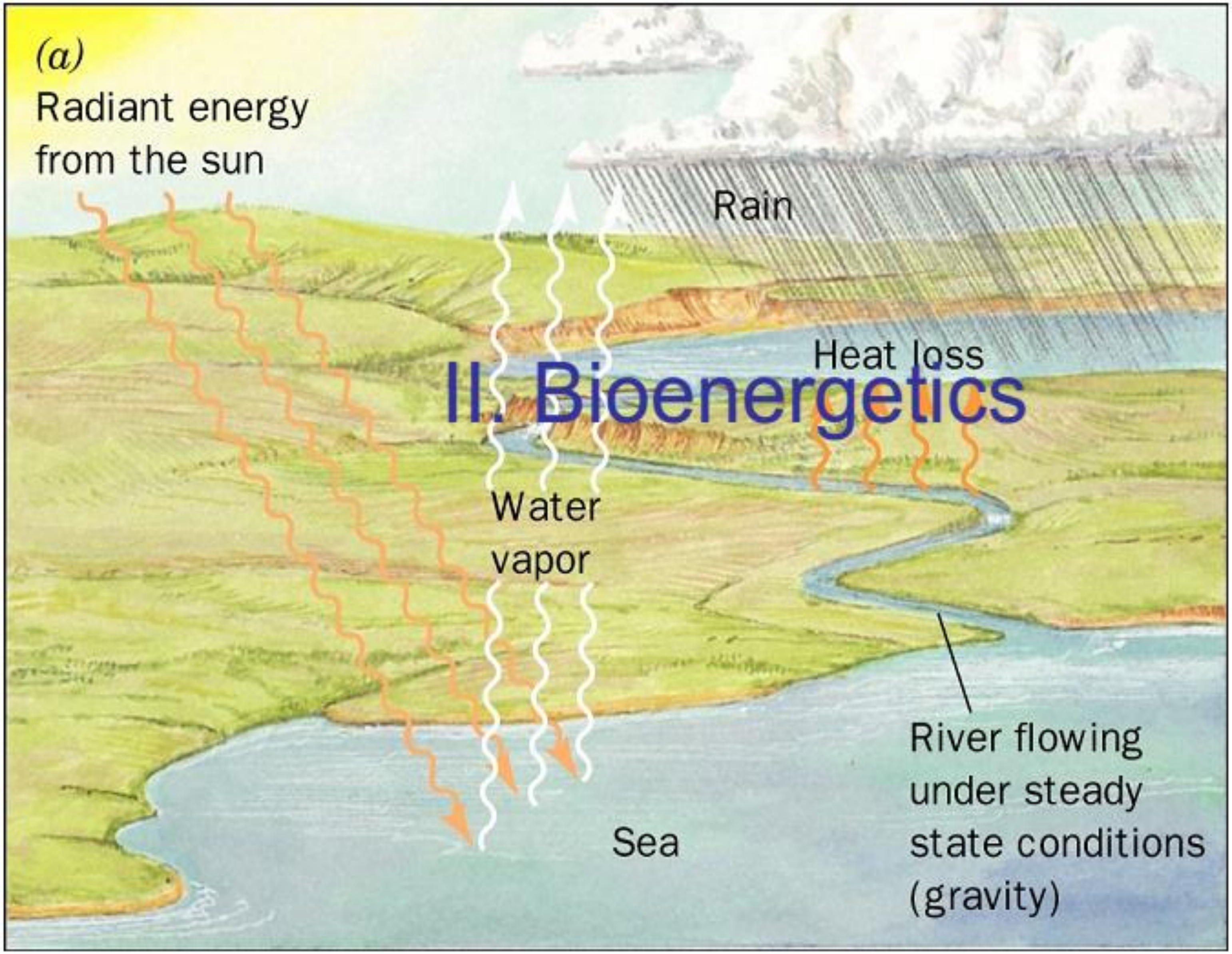
BIOENERGITICS

DR ANITHA N

Bioenergetics is the quantitative study of energy relationships and energy conversion in biological systems.

II. Bioenergetics



Energy

- Energy can exist in two states:
  - **Kinetic energy** – energy of motion.
  - **Potential energy** – stored energy.
    - Chemical energy – potential energy stored in bonds, released when bonds are broken.
- Energy can be transformed form one state to another.

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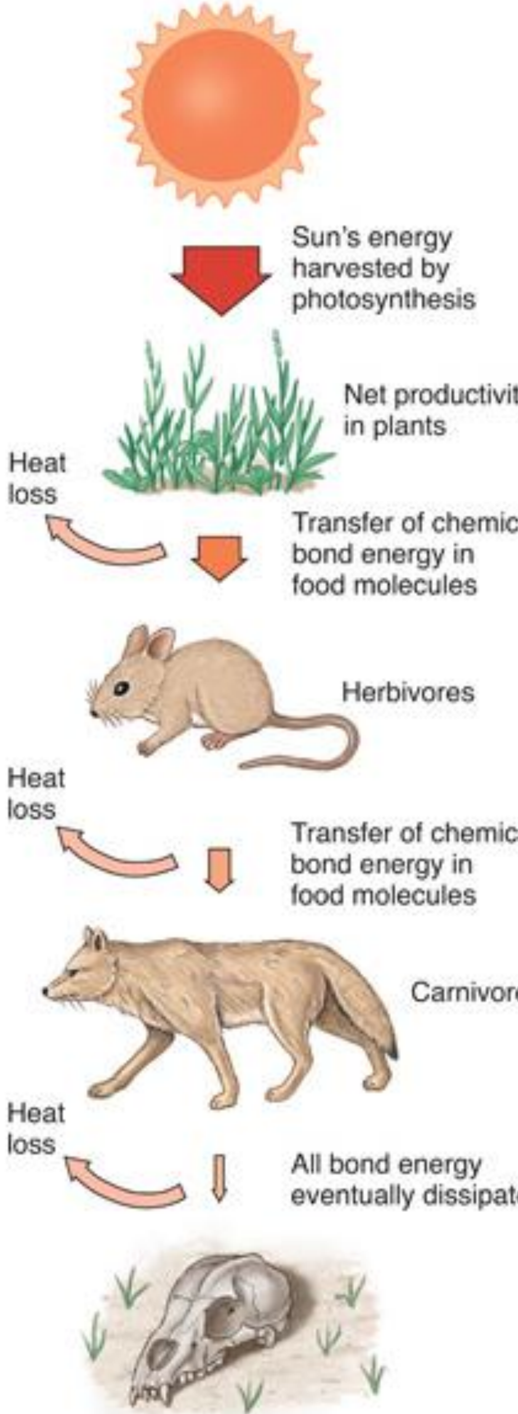

A diver has more potential energy on the platform than in the water.

Diving converts potential energy to kinetic energy.

Climbing up converts the kinetic energy of muscle movement to potential energy.

A diver has less potential energy in the water than on the platform.

Different forms of energy: Mechanical (kinetic and potential)
Light, sound, heat, electrical (including magnetic) and chemical energy

- ✓ Conversion of mass and energy
Inter-conversion of subatomic particle.
Various energy forms: Light, electromagnetic,
Kinetic, heat, potential, sound and wave

Physics

- ✓ Interactions between atoms through electrons
in outer orbit of atoms at various temperature,
pressure and pH and solvent condition.

Chemistry

- ✓ Interaction between macromolecules,
atoms in similar way as in chemistry except
at normal temperature, pressure,
around neutral pH and strictly in aqueous
Media.

Biochemistry

The Laws of Energy Transformation

- **Thermodynamics** is the study of energy transformations
- A isolated system, is isolated from its surroundings
- In an open system, energy and matter can be transferred between the system and its surroundings
- Organisms are open systems

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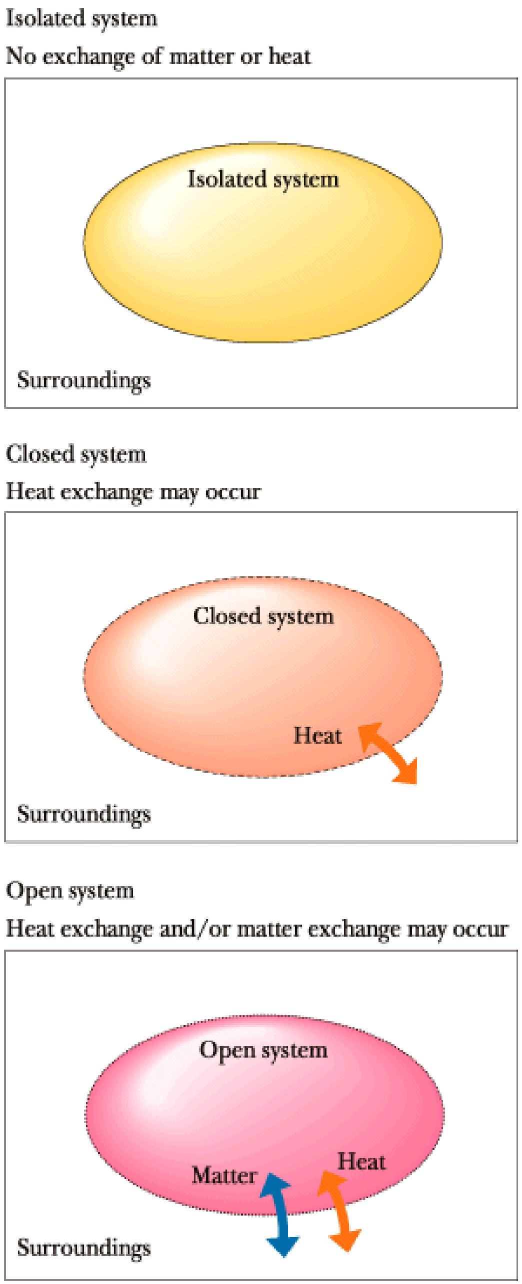
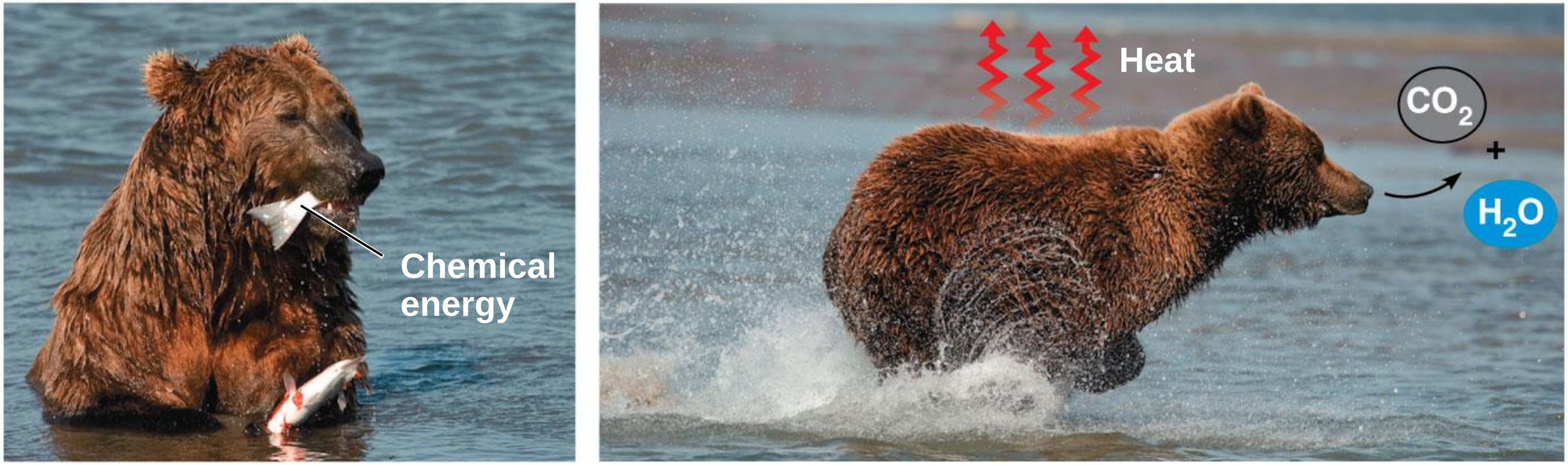


The First Law of Thermodynamics

- According to the **first law of thermodynamics**, the energy of the universe is constant
  - *Energy can be transferred and transformed, but it cannot be created or destroyed*
- The first law is also called the principle of conservation of energy

The Second Law of Thermodynamics

- During every energy transfer or transformation, some energy is unusable, and is often lost as heat
- According to the **second law of thermodynamics**
  - *Every energy transfer or transformation increases the* ***entropy*** *(disorder) of the universe*



(a) First law of thermodynamics

(b) Second law of thermodynamics

Disorder happens spontaneously

Organization requires energy

(both): © Spencer Grant/Photo Edit

Energy in chemical reactions:

Free energy (G): *It is an amount of energy capable of doing work during a reaction at constant temperature and pressure.*

- ✓ Release of free energy during the reaction is **exergonic** reaction (ΔG negative)
- ✓ Gain of free energy is **endergonic** reaction (ΔG is positive).

Enthalpy (H): *Represents the heat content of the reacting systems*.

- ✓ Release of heat during a reaction is **exothermic** reaction (ΔH is negative).
- ✓ Gain of heat during reaction is **endothermic** reaction (ΔH positive).

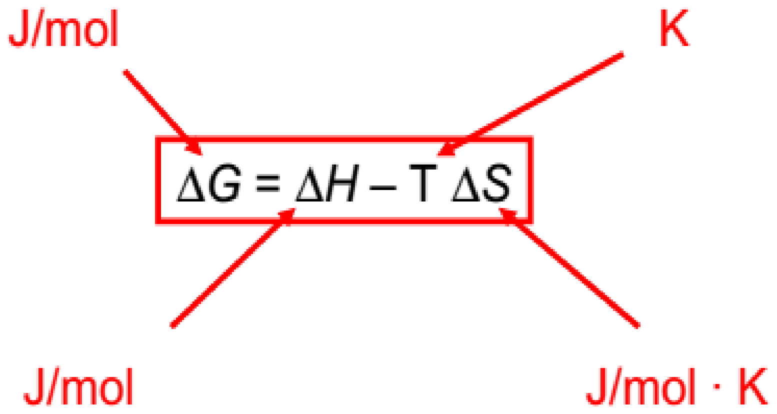
Entropy (S): it is a quantitative expression for the randomness or disorder in the system.

There is always an increase in entropy or gain of entropy of the overall system in any reaction, i.e ΔS is always +ve.

Gibbs free energy, *G* – amount of energy capable of doing work

Enthalpy, *H* – the heat content of the reacting system

Entropy, *S* – quantitative expression for the randomness or disorder in a system.



J/mol

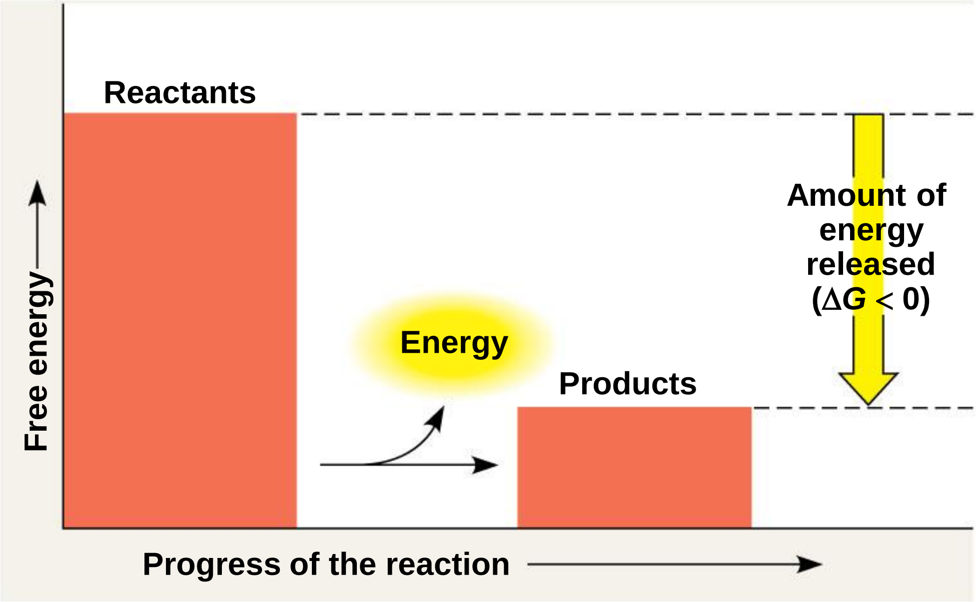
K

$$\boxed{\Delta G = \Delta H - T\,\Delta S}$$

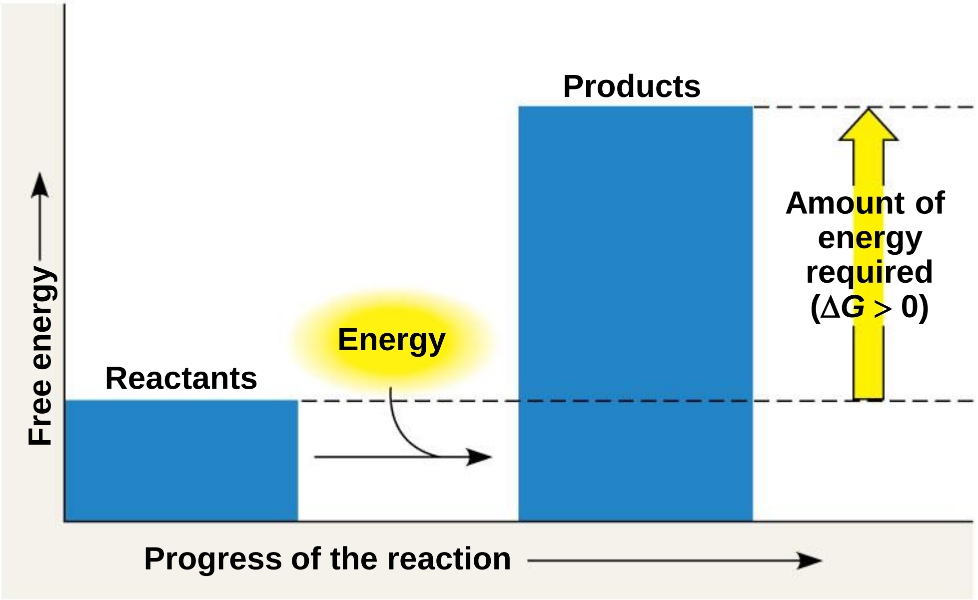
J/mol

J/mol · K

(a) Exergonic reaction: energy released, spontaneous



(b) Endergonic reaction: energy required, nonspontaneous



- ✓ The **standard Free Energy change** of a reaction is an alternative mathematical way of expression of equilibrium constant.
- ✓ It is defined as the Free energy change of a reaction at standard conditions (at 298 K and 1 atm pressure when the initial concentrations of reactants and products are at 1M each.

aA+bB = cC+dD

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

$$\boxed{\Delta G'^{\circ} = -RT \ln K'_{\text{eq}}}$$

Actual free energy change depends on reactants and product concentration

$$\boxed{\Delta G = \Delta G'^{\circ} + RT \ln \frac{[\mathrm{C}][\mathrm{D}]}{[\mathrm{A}][\mathrm{B}]}}$$

- **Standard free energy changes are additive.**

In the case of two sequential chemical reactions,

$$A \rightleftharpoons B \qquad \Delta G'^{0}_{1}$$

$$B \rightleftharpoons C \qquad \Delta G'^{0}_{2}$$

- Since the two reactions are sequential, we can write the overall reaction as

$$A \rightleftharpoons C \qquad \Delta G'^{0}_{total}$$

- The $\Delta G'^{0}$ values of sequential reactions are additive.

$$\Delta G'^{0}_{total} = \Delta G'^{0}_{1} + \Delta G'^{0}_{2}$$

$$\begin{array}{lll} & A \longrightarrow B & \Delta G'^{0}_{1} \\ & B \longrightarrow C & \Delta G'^{0}_{2} \\ \hline \text{Sum:} & A \longrightarrow C & \Delta G'^{0}_{1} + \Delta G'^{0}_{2} \end{array}$$

- This principle of bioenergetics explains how a thermodynamically unfavorable (endergonic) reaction can be driven in the forward direction by coupling it to a highly exergonic reaction through a common intermediate

The main rule in biochemical reactions in living organisms:

✓ All endergonic reactions are coupled to an exergonic reaction. There is an energy cycle in cells that links anabolic and catabolic reactions.

An example: The first step of glycolysis

- $\text{Glucose} + P_i \longrightarrow \text{Glucose 6-phosphate} + H_2O$

  $\Delta G'^0 = 13.8$ kj/mol

- $\Delta G'^0 > 0$ reaction is not spontaneous

Another very exergonic cellular reaction:

- ✓ Hydrolysis of ATP

$$\text{ATP} + H_2O \longrightarrow \text{ADP} + P_i$$

$$\Delta G'^0 = -30.5 \text{ kj/mol}$$

These two reactions share the common intermediates H_2O and P_i and may be expressed as sequential reactions:

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- ✓ Glucose + $P_i \longrightarrow$ Glucose 6-phosphate+H_2O
- ✓ ATP + $H_2O \longrightarrow$ ADP + P_i

Glucose+ATP $\longrightarrow$ Glucose 6-phosphate+ADP

The overall standard free energy changes:

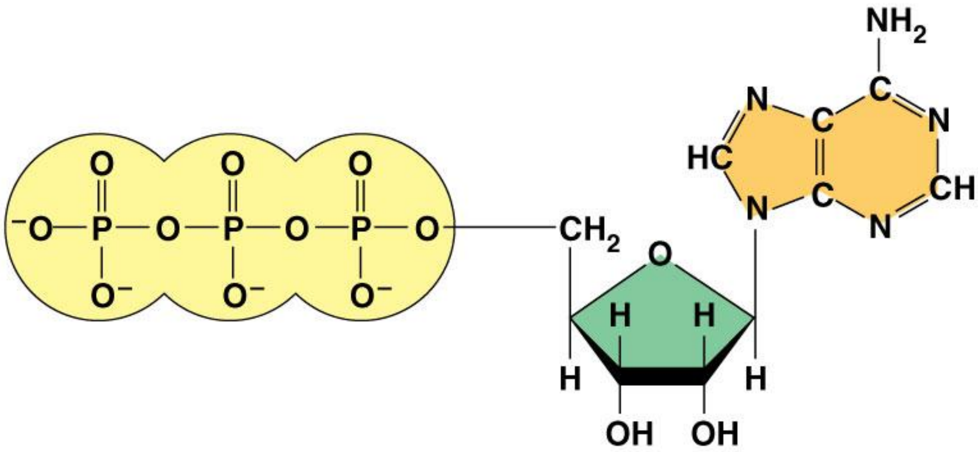
$\Delta G'^{0}$ =13.8 kj/mol + (-30.5 kj/mol)= **-16.7 kj/mol**

Overall reaction is exergonic

- ❖ Energy stored in ATP is used to drive to synthesis of glycose 6-phosphate, eventhough its formation from glucose and P_i is endergonic.
- ❖ This strategy works only if compounds such as ATP are continuously available.

ATP powers cellular work by coupling exergonic reactions to endergonic reactions

- A cell does three main kinds of work
  - -Chemical
  - -Transport $\quad$ ATP $\longrightarrow$ ADP+ P_i $\quad$ or
  - -Mechanical $\quad$ ATP $\longrightarrow$ AMP+ 2 P_i
- To do work, cells manage energy resources by **energy coupling**, the use of an exergonic process to drive an endergonic one
- Most energy coupling in cells is mediated by ATP



Phosphoryl group transfer and ATP as currency of free energy

ATP is generated during catabolic reaction and the free energy of ATP hydrolysis is used in synthetic reactions, to do mechanical work or to conduct neuronal action potential.

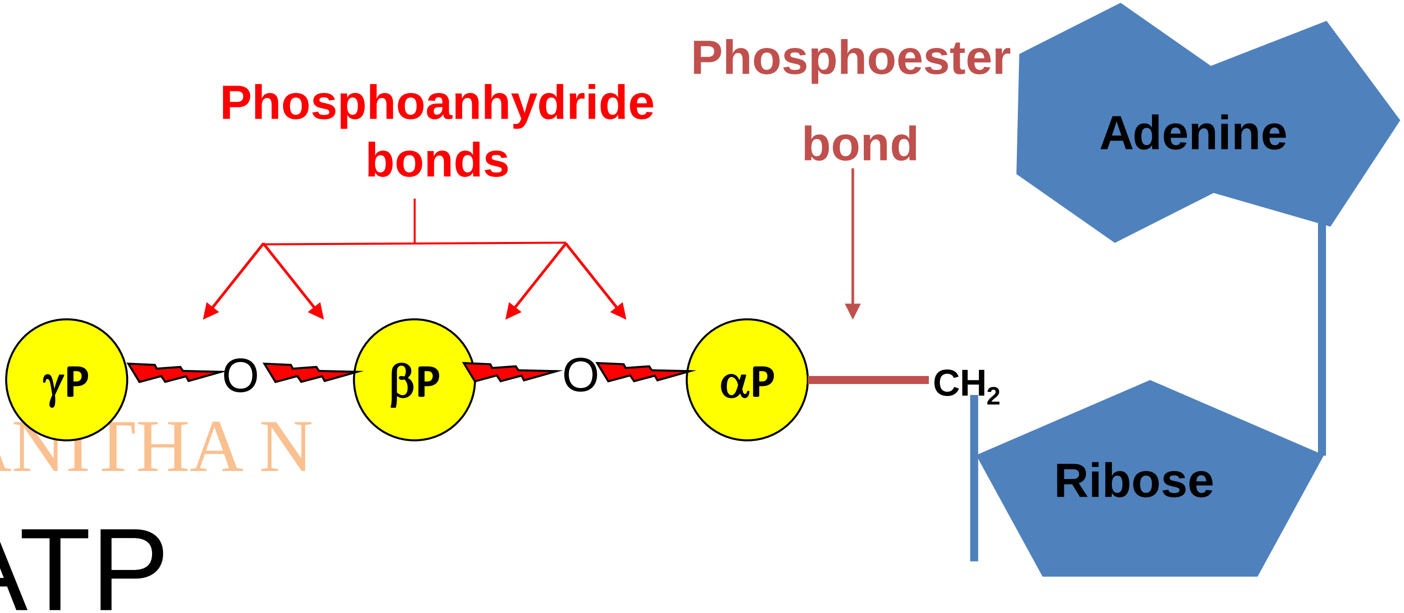
Basis of high energy of hydrolysis of ATP:

1. Resonance stabilization of products
2. Electrostatic repulsion between negatively charged oxygen atoms in ATP
3. High solvation energy of products

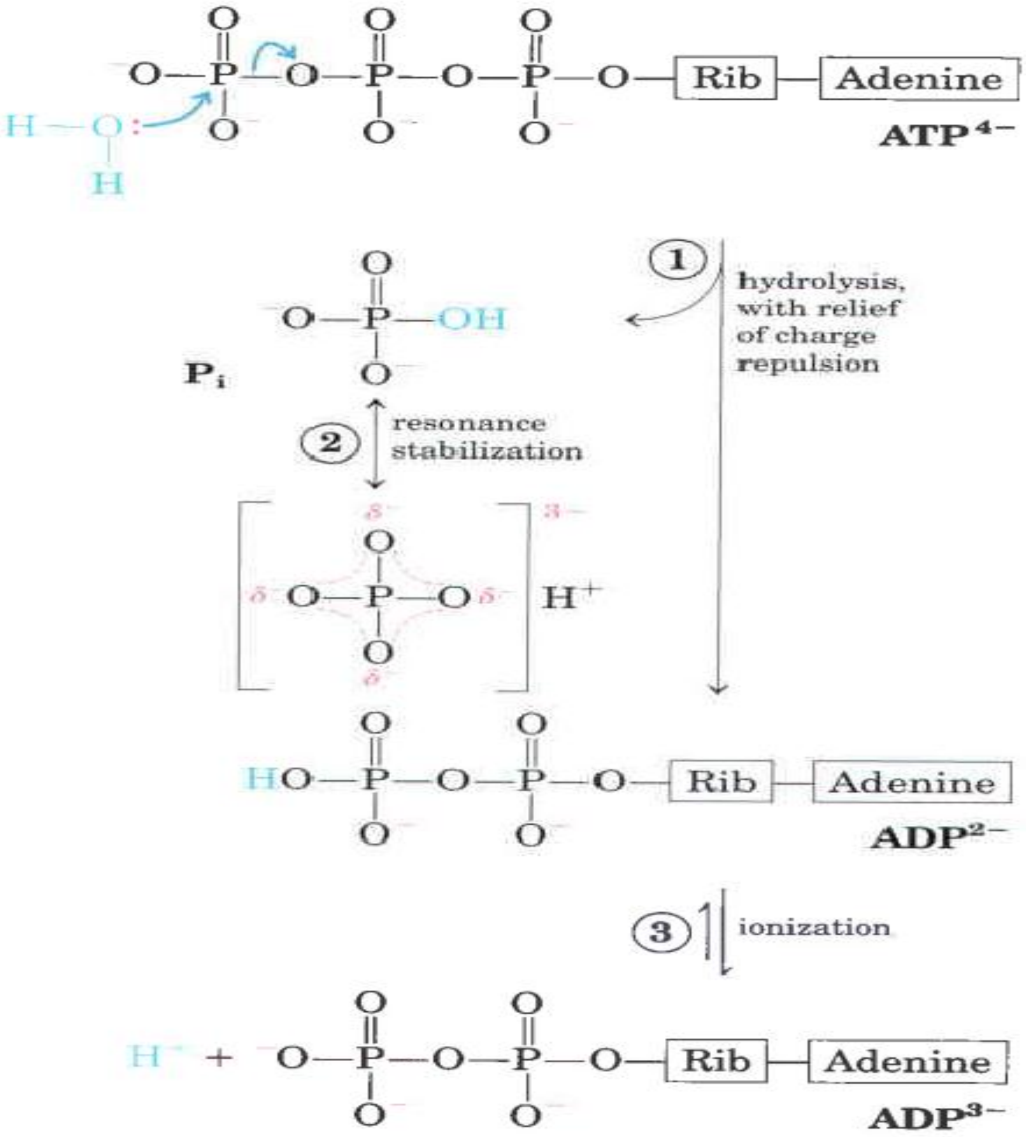
ATP is more stable than other phosphoanhydride compound

What makes the bonds in ATP ‘high-energy”?

- **Phosphoanhydride bonds** tend to have a **large negative ΔG** (-30.5 kJ.mol^{-1})



ATP



$$ATP^{4-} + H_2O \longrightarrow ADP^{3-} + P_i^{2-} + H^+$$
$$\Delta G'^{\circ} = -30.5 \text{ kJ/mol}$$

1. PhAnH bond has less stable resonance than its product

- Two strongly e$^-$ withdrawing groups compete for p e$^-$ of the bridging oxygen

hydrolysis

- No such competition in the hydrolysis product→ more stable

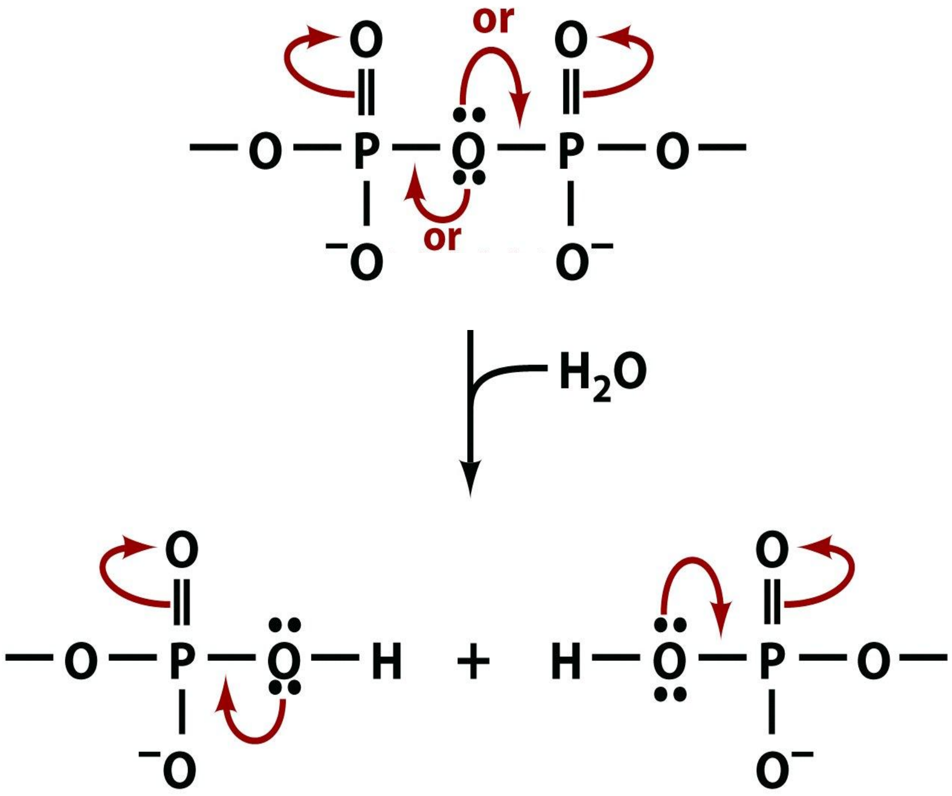


Figure 13-4 Fundamentals of Biochemistry, 2/e
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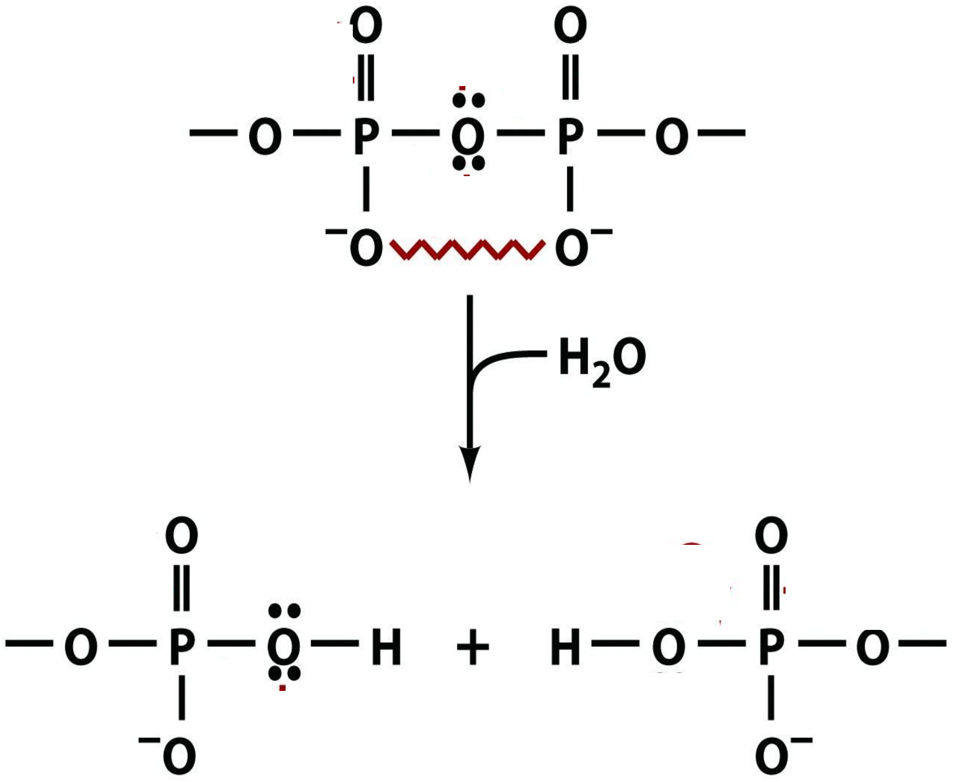
2. PhAnH bond has greater electrostatic repulsion than its product

- At pH 7, ATP has 3 –ve charges

hydrolysis

- Repulsion is relieved by hydrolysis

  → more stable



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3. Solvation energy

- Phosphoanhydride bond has smaller solvation energy than product

  → favours hydrolysis

- The free energy change for ATP hydrolysis is -30.5 kj/mol under standard conditions but the actual free energy change (ΔG) of ATP hydrolysis in living cells is very different.
- The cellular concentrations of ATP, ADP and P_i are not same and are much lower than the 1 M standard conditions.
- In addition, Mg^{2+} in the cytosol binds to ATP and ADP and for most enzymatic reactions that involve ATP as phosphorly group donor, the true substrate is $MgATP^{-2}$. The relevant $\Delta G'^0$ is therefore that for $MgATP^{-2}$ hydrolysis.

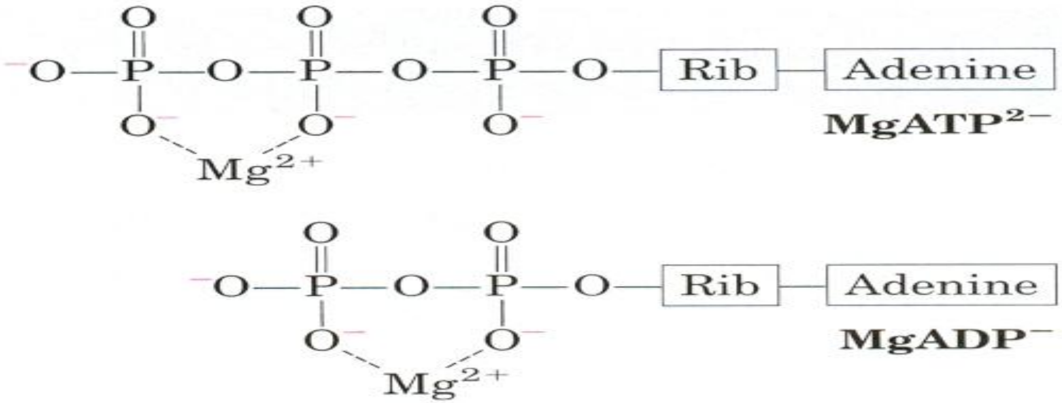

FIGURE 13–2 **Mg^{2+} and ATP.** Formation of Mg^{2+} complexes partially shields the negative charges and influences the conformation of the phosphate groups in nucleotides such as ATP and ADP.

Phosphoryl group-transfer potential

- Measure of tendency of compound to transfer ~P to H_20
- ATP is intermediate!
- Can accept ~P from compounds above
- Or donate ~P to compounds below

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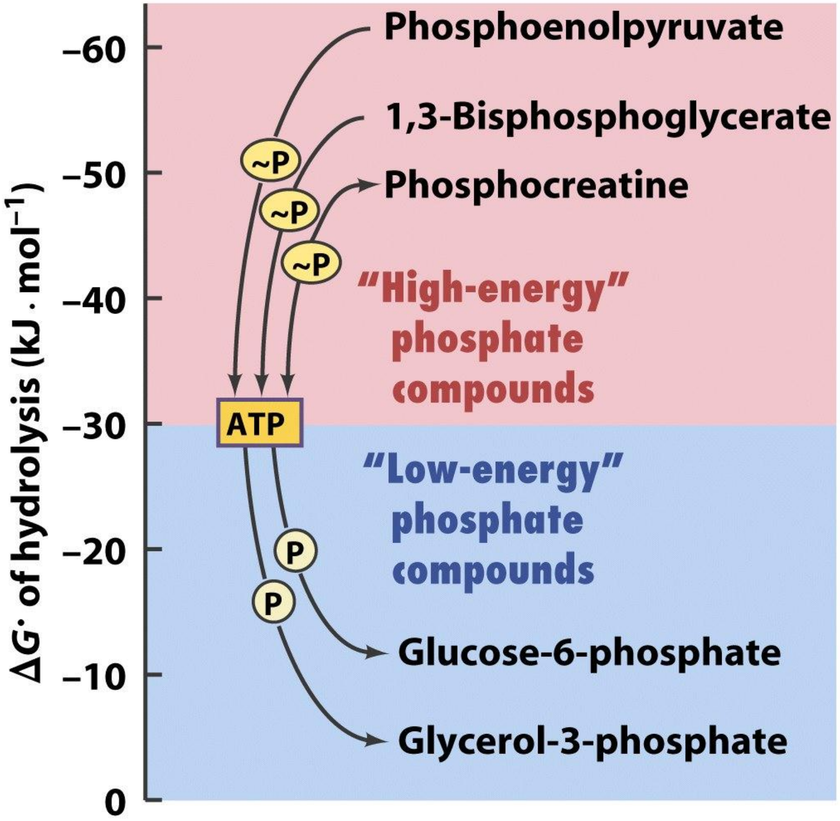


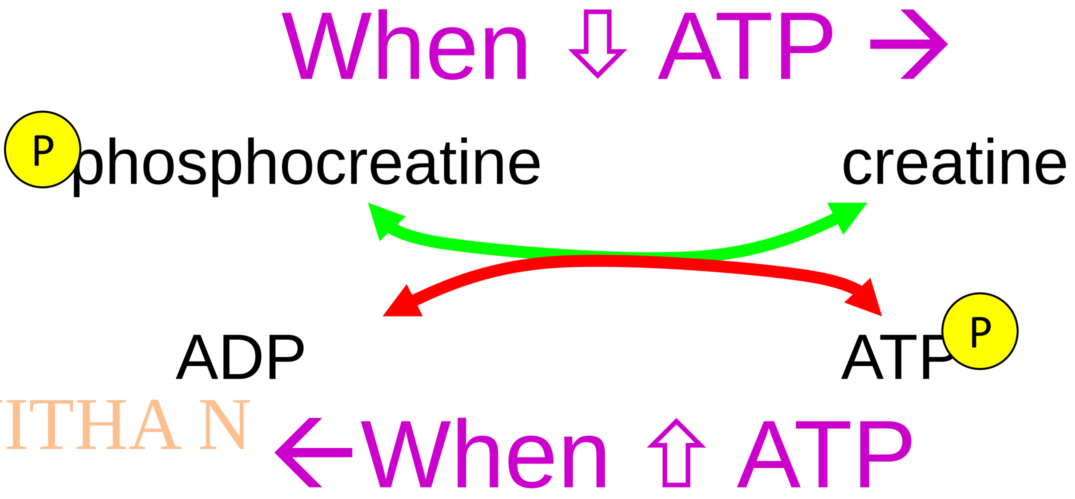
Figure 13-7 Fundamentals of Biochemistry, 2/e

Phosphocreatine

- Higher P-group transfer potential than ATP

- 'reservoir' of ~P for rapid ATP regeneration →Maintains constant level of ATP by swapping ~P

  =reversible 'substrate-level phosphorylation' in tissues with high need (muscle, nerve)



When ⇩ ATP →

P phosphocreatine

creatine

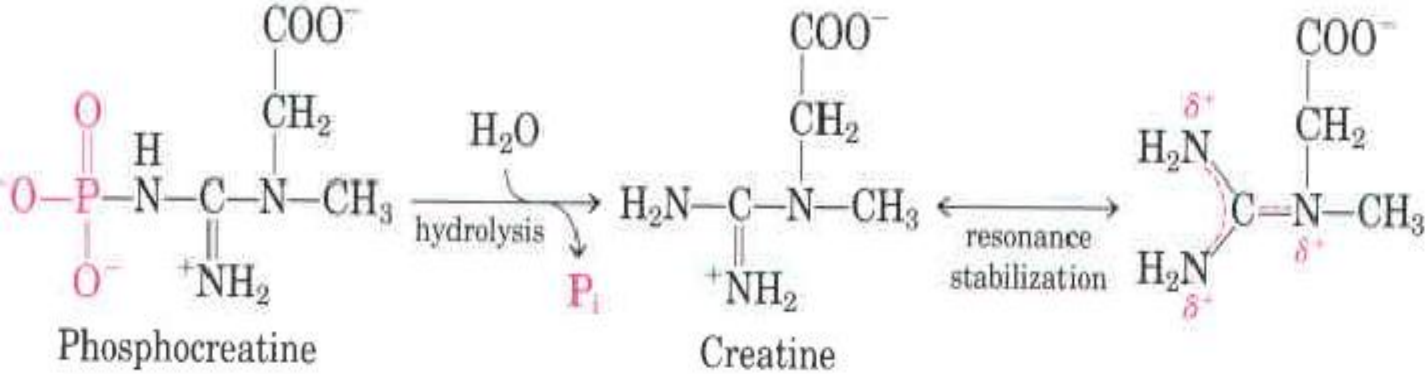
ADP

ATP P

←When ⇧ ATP

When ATP is low, phosphocreatine can lend a P to ADP to make ATP.

When ATP is replenished by catabolism, P is 'paid back".

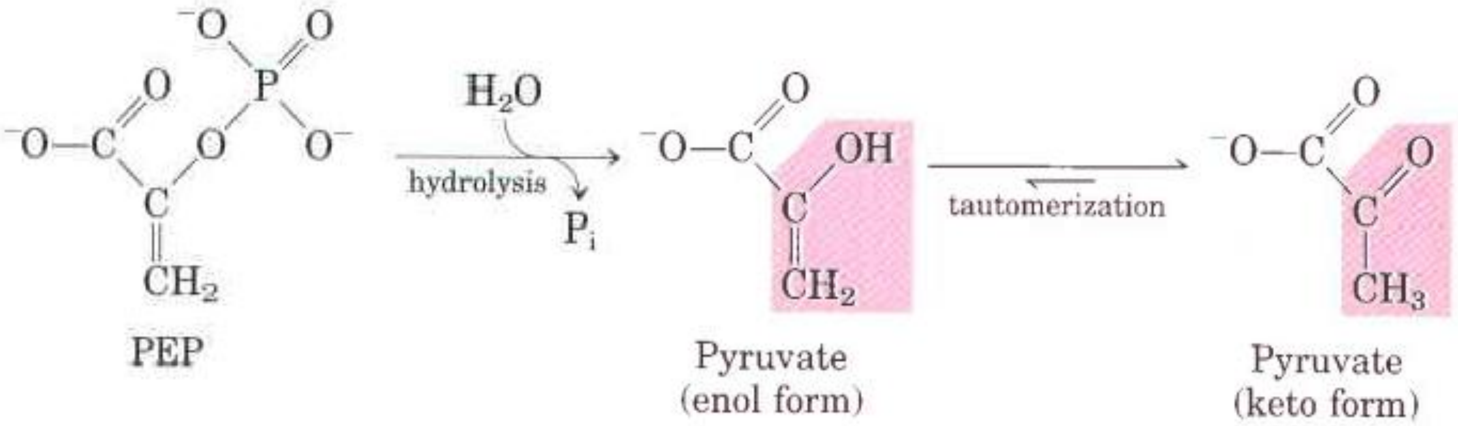


$$\text{Phosphocreatine}^{2-} + H_2O \longrightarrow \text{creatine} + P_i^{2-}$$

$$\Delta G'^{\circ} = -43.0\ \text{kJ/mol}$$

Phosphoenol pyruvate

- Phosphoenolpyruvate contains a phosphate ester bond that undergoes to yield to enol form of pyruvate
- The enol form of pyruvate can immediately tautomerize to the more stable keto form of pyruvate. Because phosphoenolpyruvate has only one form (enol) and the product, pyruvate, has two possible forms, the product is more stabilized relative to the reactant.
- This is the greatest contributing factor to the high standard free energy change of hydrolysis of phosphoenolpyruvate ($\Delta G'^0$ = -61,9 kj/mol)

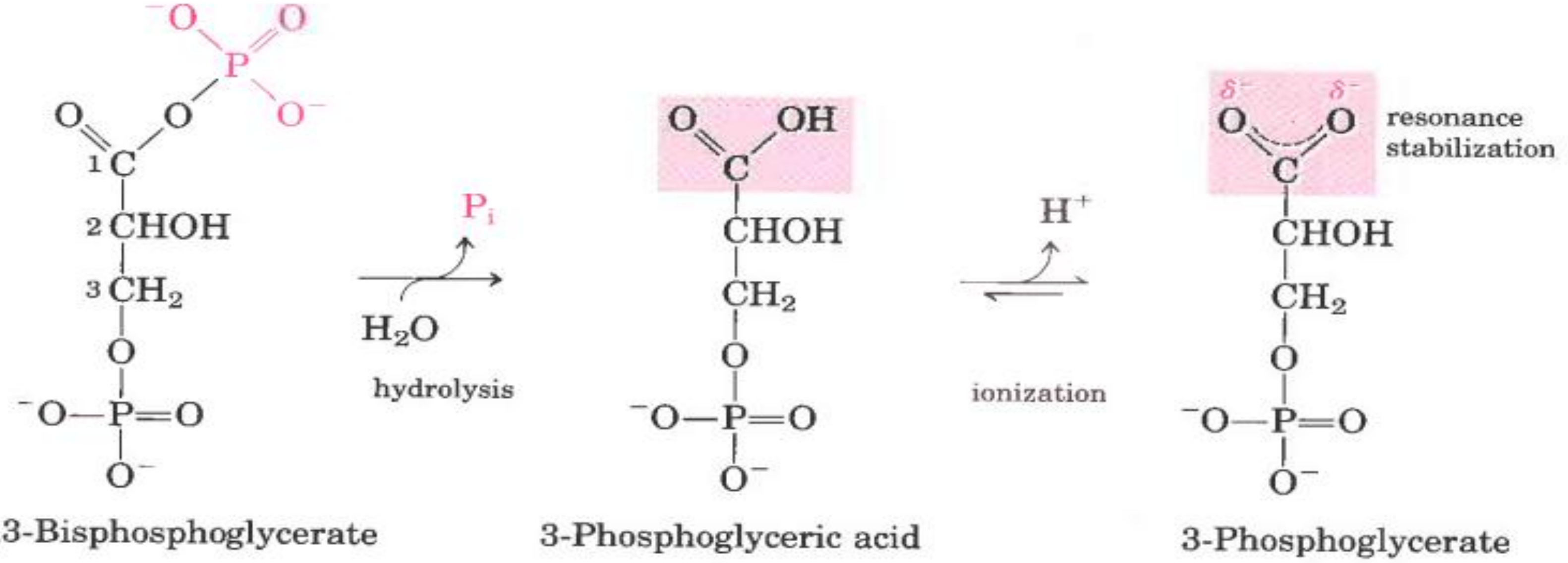


D

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

1,3-bisphosphoglycerate

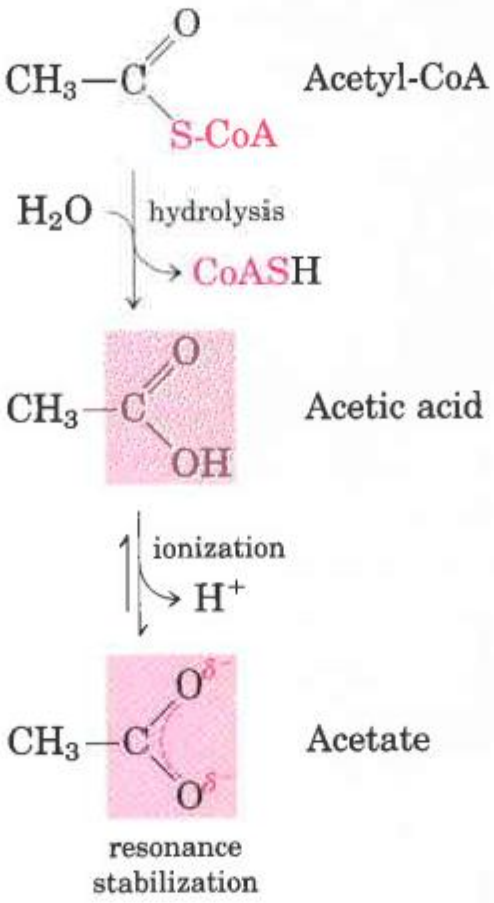
- ✓ 1,3-bisphosphoglycerate contains an **anhydride bond** between the carboxyl group at C-1 and phosphoric acid.
- ✓ Hydrolysis of this acyl phosphate is accompanied by a large, negative, standard free energy change ($\Delta G'^0 = -49{,}3$ kj/mol)
- ✓ This large, negative $\Delta G'^0$ can, again, be explained in terms of the structure of reactants and products



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

Thioesters

- In thioesters a sulfur atom is replaced the usual oxygen in the ester bond
- Thioesters have large, negative standard free energy change of hydrolysis.
- Acetyl coenzyme A is one of many thioesters important in metabolism. The acyl group in these compounds is activated for trans-acylation, condensation or oxidation-reduction reactions.
- Hydrolysis of the ester bond generates a carboxylic acid which can ionize and assume several resonance forms.
- $\Delta G'^{0} = -31.4$ kj/mol for acetyl-CoA hydrolysis



$$\text{Acetyl-CoA} + H_2O \longrightarrow \text{acetate}^- + \text{CoA} + H^+$$

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

Summary for hydrolysis reactions

- *For hydrolysis reactions with large, negative standard free energy changes, the products are more stable than the reactants for one or more of the following reasons:*

✓ 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, 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 phosphate released from anhydride or ester linkages

- The phosphate compounds found in living organisms can be arbitrarily divided into two groups based on their standard free energy changes of hydrolysis.

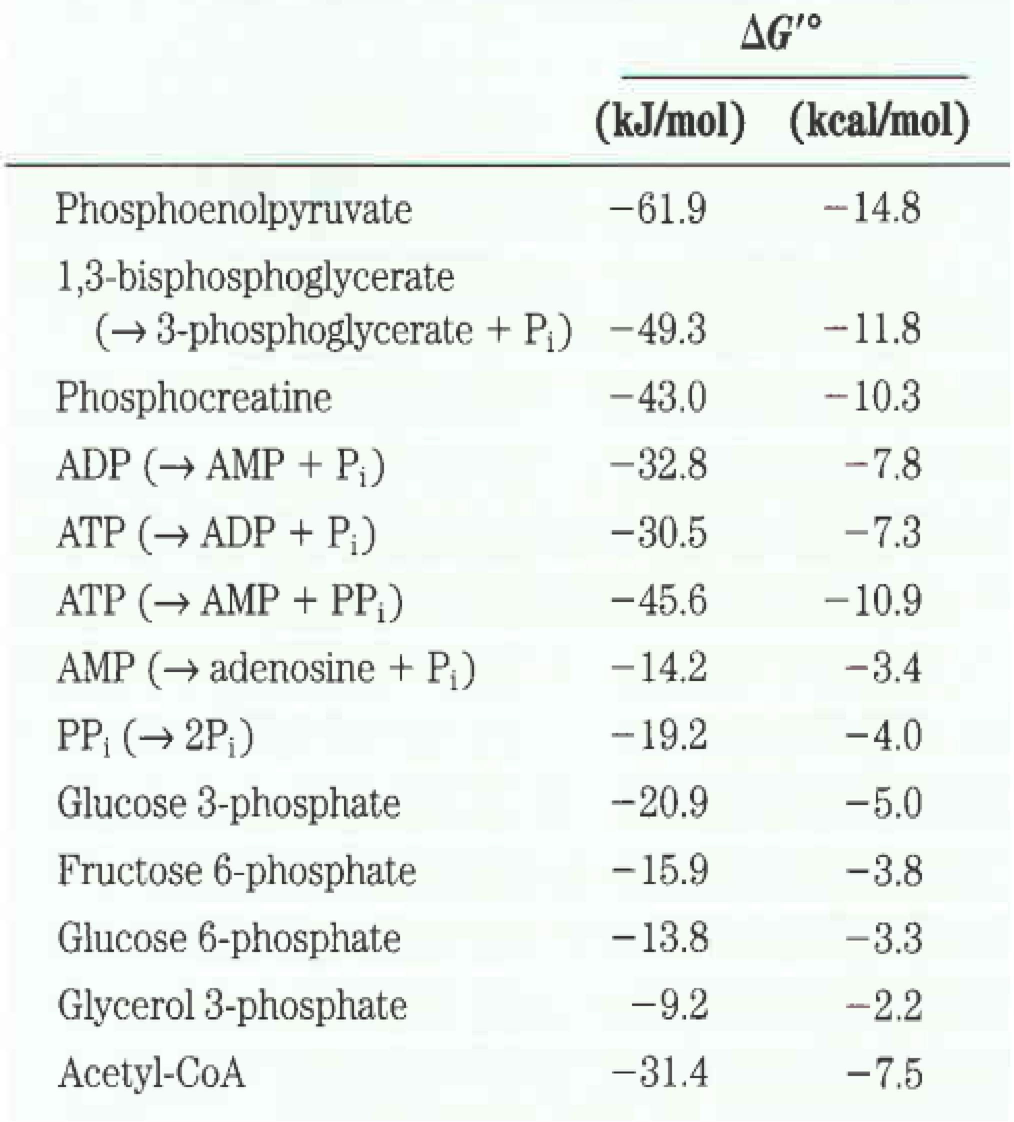
✓ ‘High-energy’ compounds have a $\Delta G'^{0}$ of hydrolysis more negative than -25 kj/mol;

ATP, with a $\Delta G'^{0}$ of hydrolysis of -30 kj/mol is a high-energy compound;

✓ ‘low-energy’ compounds have a less negative $\Delta G'^{0}$.

Glucose 6-phosphate is a low-energy compound ($\Delta G'^{0}$ = -13,8 kj/mol)

Standard free energies of hydrolysis of some phosphorylated compounds and Acetyl -CoA (thioester)



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

WHY Phosphorylated Intermediates?

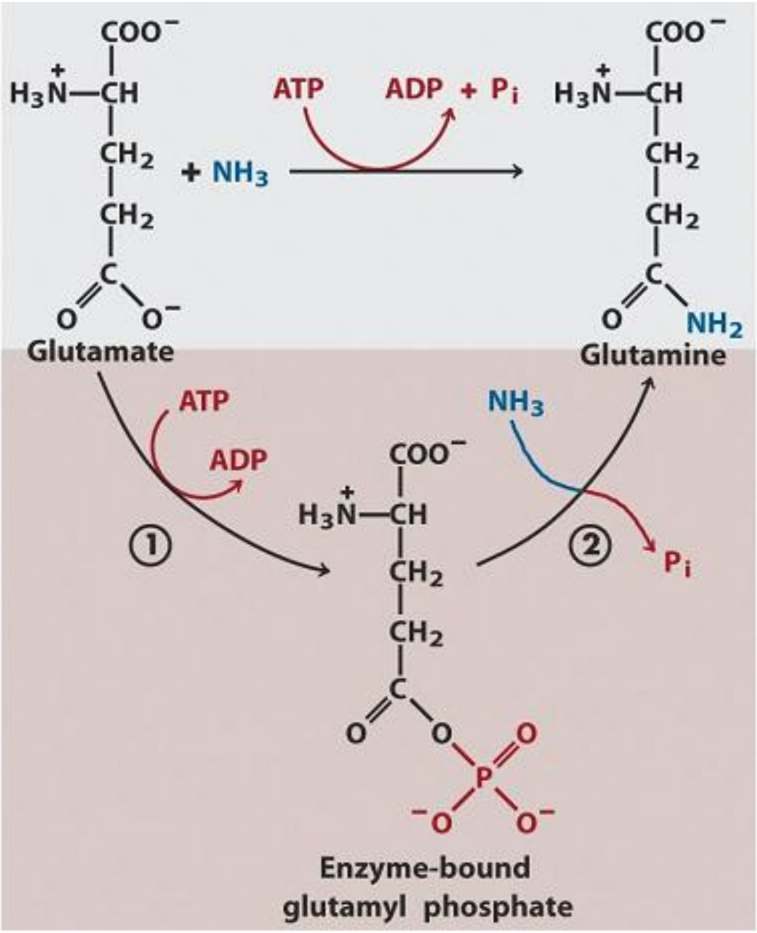
- ✓ Keeps intermediates inside cell, as phosphorylated Intermediates are negatively charged and cannot leave cell through hydrophobic membrane, and thus maintenance of high concentrations of the intermediates inside the cell is possible (even though opposed by a concentration gradient).

- ✓ Conserve the energy stored in the original ATP molecule in the phosphoanhydride bond.

- ✓ Increased binding energy for phosphorylated Intermediates on enzyme active sites lowering activation energy barrier, increasing enzyme specificity. Mg^{2+} ions are often key to this recognition process.

Group Transfer

- ✓ ATP provides energy not by simple hydrolysis. It is provided by group transfers.
- ✓ A reaction usually written as a one-step reaction may actually involve two steps.
- ✓ A phosphoryl group is transferred from ATP to a substrate (here glutamate), then the phosphoryl group is displaced by a reactant (here NH_3) resulting in the release of P_i



As is evident from the additivity of free energy changes of sequential reactions, any phosphorylated compound can be synthesized by coupling the synthesis to the breakdown of another phosphorylated compound with a more negative standard free energy change of hydrolysis.

Example:

$\Delta G'^{0}$

- $\text{PEP} + H_2O \longrightarrow \text{Pyruvate} + P_i$ -61.9
- $\text{ADP} + P_i \longrightarrow \text{ATP} + H_2O$ +30.5

$\text{PEP} + \text{ADP} \longrightarrow \text{Pyruvate} + \text{ATP}$ -31.4

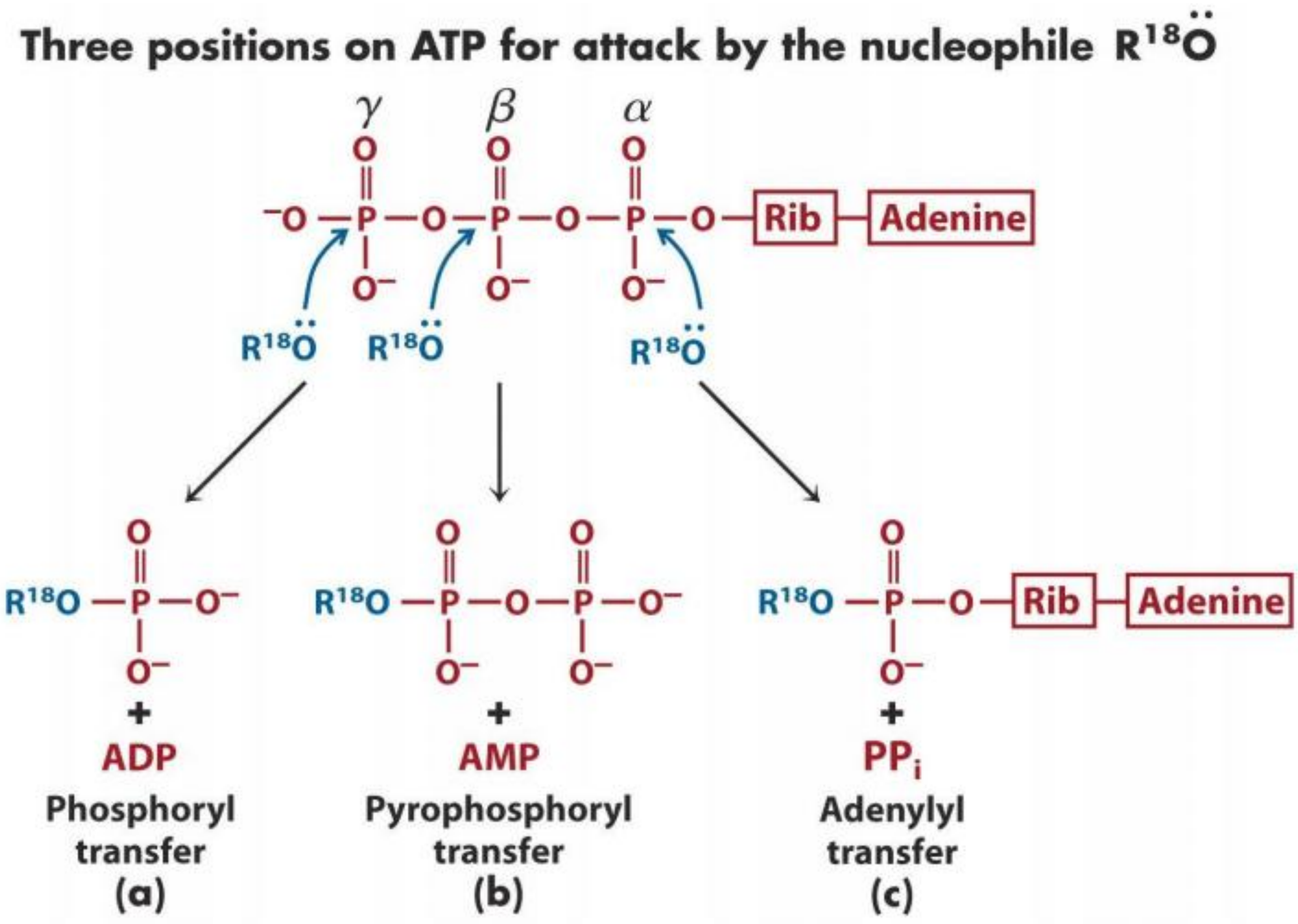
Clevage of P_i from PEP releases more energy than is needed to drive to condensation of P_i with ADP, the direct donation of a phosphoryl group from PEP to ADP is thermodynamically feasible.

- One more chemical feature of ATP is crucial to its role in metabolism: although in aqueous solution ATP is thermo-dynamically unstable and is therefore a good phosphoryl group donor, it is kinetically stable.

- Because of high activation energies required for uncatalyzed reaction ATP does not spontaneously donate phosphoryl groups to water or to the other potential acceptors in the cell.

- Kinetic stability is essential feature of “energy storage” molecules
  • Rapid ATP hydrolysis in the absence of a catalyst would render ATP useless as an
-

  energy storage molecule as it would fall apart before use
  • Allows for ATP hydrolysis only when reaction is coupled to a useful cellular reaction



DR A

$$ATP + 2H_2O \longrightarrow AMP + 2P_i \qquad \Delta G'^\circ = -64.8\,\mathrm{kJ/mol}$$

Role of "high energy" bonds:

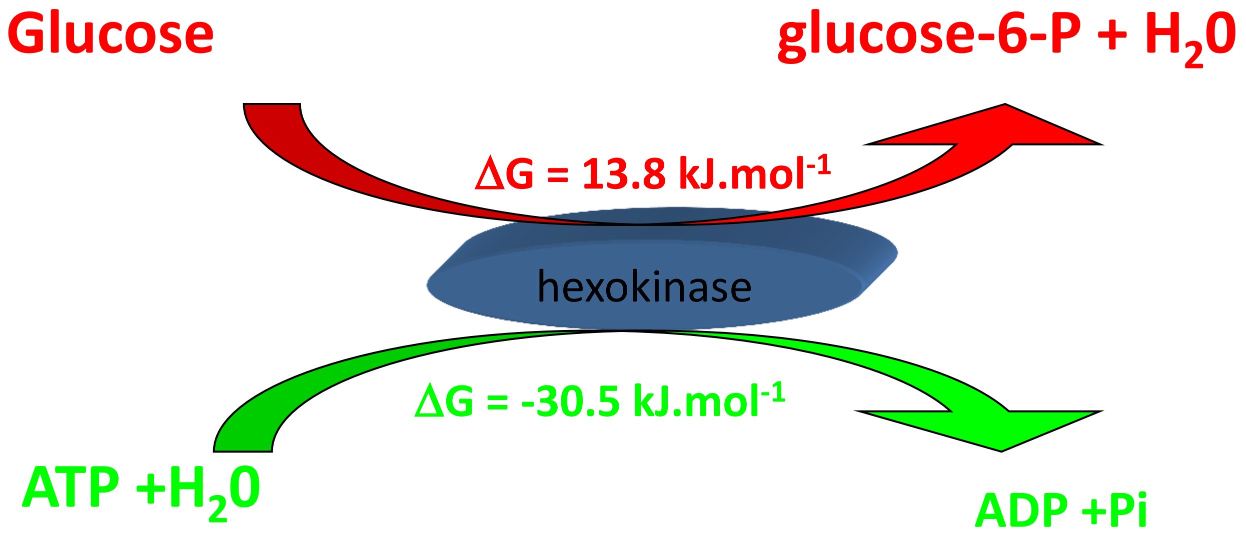
- **Energy transfer or storage**
  ATP, PP_i, polyphosphate, phosphocreatine
- **Group transfer**
  ATP, Coenzyme A
- **Transient signal**
  cyclic AMP

Why create high energy compounds?

- **spontaneous reactions** $\Delta G<0$ **are often coupled with non-spontaneous reactions** $(\Delta G>0)$ **to drive them forward.**
- The free-energy change (ΔG) for **coupled** reactions is the **sum** of the free-energy changes for the **individual reactions**.

$$\Delta G_{\text{coupled}} = \Delta G_{\text{reaction 1}} + \Delta G_{\text{reaction 2}}$$

- **Thus, ATP → ADP +Pi ($\Delta G<0$) is coupled with non-spontaneous reactions ($\Delta G>0$) to drive them forward.**



Glucose

glucose-6-P + H_20

$\Delta G = 13.8\ kJ.mol^{-1}$

hexokinase

$\Delta G = -30.5\ kJ.mol^{-1}$

ATP +H_20

ADP +Pi

$$\text{Glucose + ATP} \xrightarrow[\text{Overall: spontaneous!}]{\Delta G = -16.3\ kJ.mol^{-1}} \text{glucose-6-P + ADP}$$

Transphosphorylations between nucleotides

- Although we have focused on ATP as the cell’s energy currency and donor of phosphoryl groups.
- All other nucleoside triphosphates (GTP, UTP, CTP) and all the deoxynucleoside triphosphates (dATP, dGTP, dTTP and dCTP) are energetically equivalent to ATP. The free energy changes associated with hydrolysis of their phosphoanhydride linkages are very nearly identical with those for ATP.
- In preparation for their various biological roles, these other nucleotides are generated as the nucleoside triphosphate (NTP) forms by phosphoryl group transfer to the corresponding nucleoside diphosphates (NDPs) and monophosphates (NMPs)
- ATP is the primary high-energy phosphate compound produced by catabolism in the processes of glycolysis, oxidative phosphorylation. Several enzymes carry phosphoryl groups from ATP to the other nucleotides

- Nucleoside diphosphate kinases, found in all cells, catalyzes the reaction

$$\text{ATP+NDP(or dNDP)} \overset{\text{Mg}^{2+}}{\rightleftharpoons} \text{ADP+NTP(or dNDP)}$$

- Although this reaction is fully reversible the relatively high ATP/ADP ratio in cells normally drives the reaction to the right, with the net formation of NTPs and dNTPs

- Phosphoryl group transfers from ATP result in an accumulation of ADP.

For example, when muscle is contracting vigorously ADP accumulates and interferes with ATP-dependent contraction.

- During periods of intense demand for ATP, the cell lowers the ADP concentration, and at the same time acquires ATP, by the action of adenylate kinase:

$$2ADP \overset{Mg^{2+}}{\leftrightarrows} ATP + AMP$$

Phosphocreatine (PCr) serves as a ready source of phosphoryl groups for the quick synthesis of ATP from ADP. The phosphocreatine concentration in skeletal muscle is considerably higher than those in the other tissues. The enzyme creatine kinase catalyzes the reversible reaction.

$$ADP + PCr \overset{Mg^{2+}}{\leftrightarrows} ATP + Cr$$

$$\Delta G'^{0} = -12.5 \text{ kj/mol}$$