Biochemistry II (BB 422/622)

Review of 421
Goals of 422
Review of chemical principles
    Thermo
    C/O cycles
    Overview of Metabolism
    ATP cycles
Energy Coupling
Chemical Reactivity } Common types of organic chemistry reaction mechanisms
Bioenergetics
Membrane transport

Energetics of Life

The **laws of thermodynamics** apply to all matter and all energy transformations in the Universe.

<u>1st & 2nd Laws of Thermodynamics:</u>

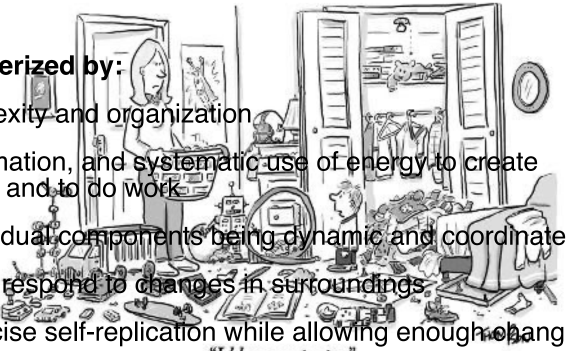
1) Energy can never be created or destroyed, but can be interconverted.

2) The universe tends toward more disorder (randomness)

[When energy is converted from one form to another, some of that energy becomes unavailable to do work.]

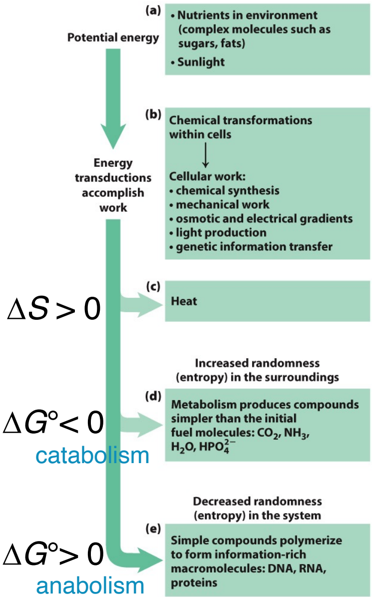
Living matter is characterized by:

- a high degree of complexity and organization
- the extraction, transformation, and systematic use of energy to create and maintain structures and to do work
- the interactions of individual components being dynamic and coordinated
- the ability to sense and respond to changes in surroundings
- a capacity for fairly precise self-replication while allowing enough change for evolution



"I blame entropy."

Organisms Use the First Law Big-time (performing energy transformations) to Stay Alive



Potential energy

(a)
- Nutrients in environment (complex molecules such as sugars, fats)
- Sunlight

Energy transductions accomplish work

(b) Chemical transformations within cells

Cellular work:
- chemical synthesis
- mechanical work
- osmotic and electrical gradients
- light production
- genetic information transfer

$\Delta S > 0$

(c) Heat

Increased randomness (entropy) in the surroundings

$\Delta G° < 0$

catabolism

(d) Metabolism produces compounds simpler than the initial fuel molecules: CO_2, NH_3, H_2O, HPO_4^{2-}

Decreased randomness (entropy) in the system

$\Delta G° > 0$

anabolism

(e) Simple compounds polymerize to form information-rich macromolecules: DNA, RNA, proteins

Favorable and Unfavorable Reactions

- The breakdown of some metabolites releases a significant amount of energy (**exergonic**).
  - Their cellular concentration is far higher than their equilibrium concentration.
  - Metabolites, such as ATP, NADH, NADPH, can be synthesized using the energy from sunlight and fuels…..
- Synthesis of complex molecules and many other metabolic reactions requires energy (**endergonic**).
  - A reaction might be thermodynamically unfavorable ($\Delta G° > 0$).
    - Creating order requires work and energy.

Biochemistry couples exergonic with endergonic reactions to ensure organisms continue to grow and divide.

Energetics of Life

- Living organisms cannot create energy from nothing.
- Living organisms cannot destroy energy into nothing.
- Living organism may transform energy from one form to another.
- In the process of transforming energy, living organisms must increase the entropy of the universe.
- In order to maintain organization within themselves, living systems must be able to extract useable energy from their surroundings and release useless energy (heat) back to their surroundings.

Overview of Metabolism

Metabolism

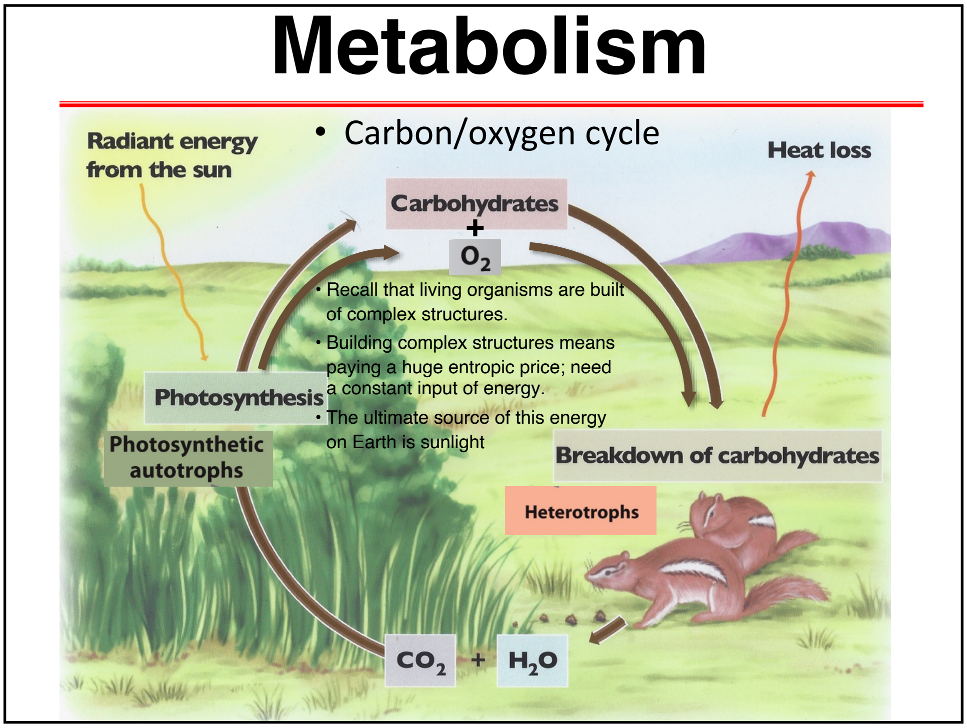
Issues:

- Thermodynamics and biochemistry; carbon/oxygen cycle & nitrogen cycle
- Common organic chemistry principles in biochemistry
- Some biomolecules are "high energy" with respect to their hydrolysis and group transfers.
- Energy stored in reduced organic compounds can be used to reduce cofactors such as NAD^+ and FAD, which serve as universal electron carriers and lead to ATP formation.

"Now, in the *second* law of thermodynamics . . . "

Sidney Harris

Unnumbered 13 p496
Lehninger Principles of Biochemistry, Seventh Edition



Metabolism

- Carbon/oxygen cycle

- Recall that living organisms are built of complex structures.
- Building complex structures means paying a huge entropic price; need a constant input of energy.
- The ultimate source of this energy on Earth is sunlight

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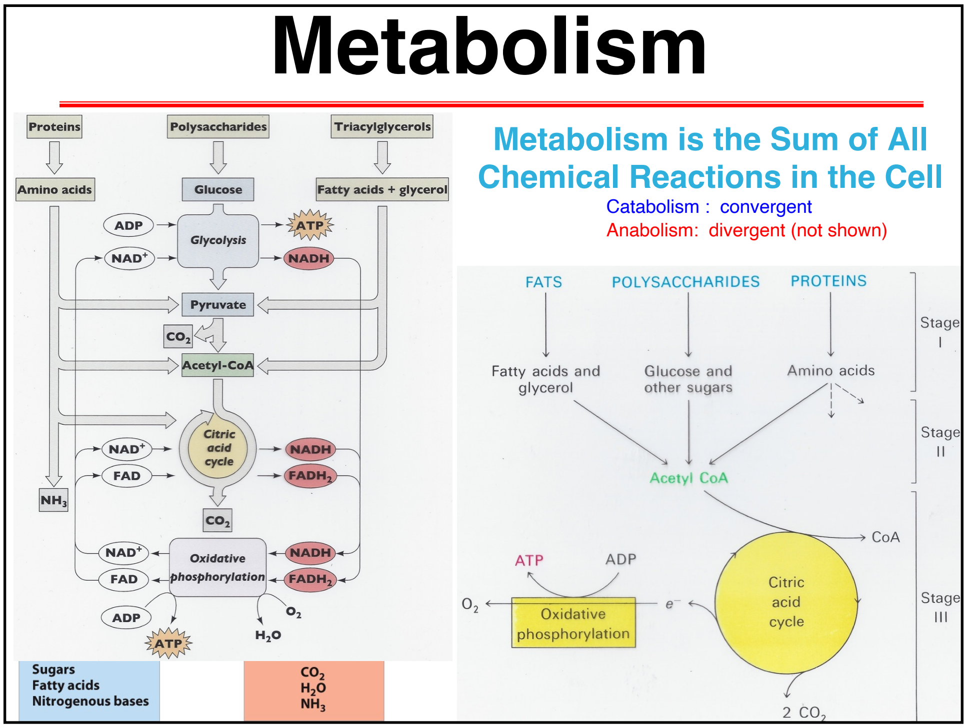

Metabolism

Metabolism is the Sum of All Chemical Reactions in the Cell

Catabolism : convergent

Anabolism: divergent (not shown)

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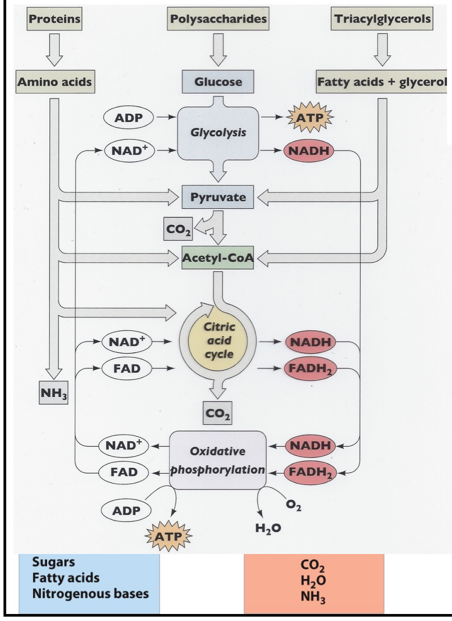
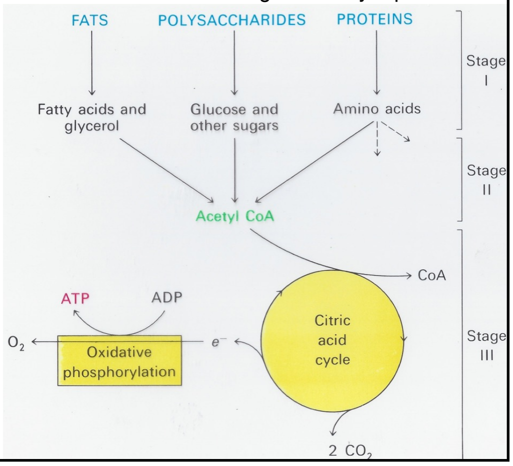
Metabolism

- In biochemistry, the oxidation of reduced fuels with O_2 is stepwise and controlled.
- Catabolism is convergent and Anabolism is divergent
- Recall that being thermodynamically favorable is not the same as being kinetically rapid.





Metabolism

NAD^+ and $NADP^+$ Are Common Redox Cofactors

- These are commonly called pyridine nucleotides.
- They can dissociate from the enzyme after the reaction.
- In a typical biological oxidation reaction, hydride ($H:^-$), from an alcohol is transferred to NAD^+, giving NADH.

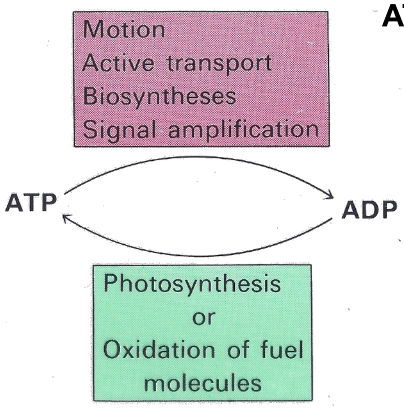
FAD and FMN are another Common Redox Cofactors

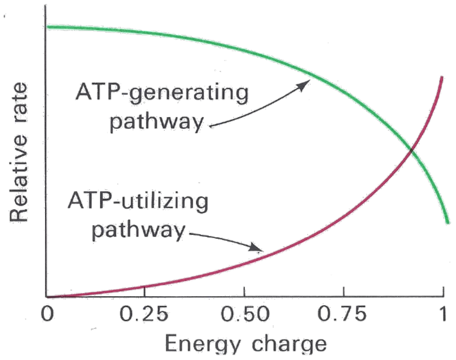
- These are commonly called flavins.
- They are usually covalently bound at the active site of enzymes.
- They can undergo both 1-electron and 2-electron redox reactions.

Metabolism

THE ATP CYCLE

$$ATP^{-4} + H_2O \rightleftharpoons ADP^{-3} + H_2PO_4^{-}$$

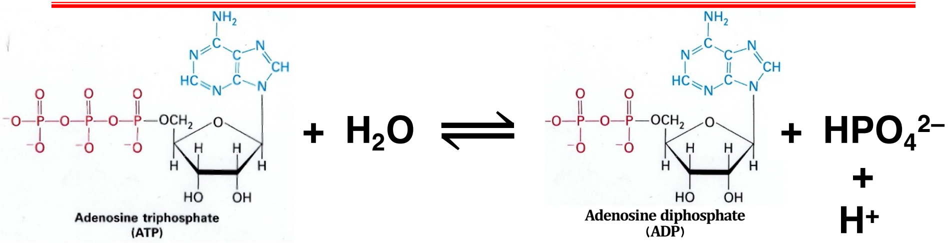




This “buffering” of energy in the cell keeps the [ATP] high enough to keep fighting the second law of thermodynamics.

$$\text{Energy Charge} = \frac{[ATP^{-4}] + \tfrac{1}{2}[ADP^{-3}]}{[ATP^{-4}] + [ADP^{-3}] + [AMP^{-2}]}$$

Metabolism



$$ATP + H_2O \rightleftharpoons ADP + HPO_4^{2-} + H^+$$

(at pH 7)

If this reaction is allowed to come to equilibrium, what is the ΔG?

Recall, at equilibrium, ΔG=0

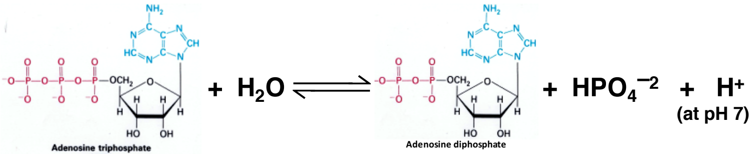
Also, recall that the actual free-energy change of a reaction depends on:

- A constant (the standard change in free energy, ΔG°)
- actual concentrations of products and reactants
- For the reaction aA + bB ⇌ cC + dD:

$$\Delta G' = \Delta G^{\circ\prime} + RT \ln \frac{[C]^c[D]^d}{[A]^a[B]^b}$$

[In biochemistry, we add a prime (′) because we pull [H+] out of the expression and set it to 10^{-7}, not 1 M]

Metabolism



At equilibrium, ΔG=0

$$\Delta G' = \Delta G°' + RT\ln\frac{[C]^c[D]^d}{[A]^a[B]^b}$$

BUT, if we are at equilibrium, this equation becomes:

$$\Delta G' = \Delta G°' + RT\ln K'_{eq}$$

$$\Delta G°' = -RT\ln K'_{eq}$$

For the above reaction (hydrolysis of ATP):

$$\boxed{\Delta G°' = -7.3\ \text{kcal/mole}}$$

TABLE 13-3 Relationships among K'_{eq}, $\Delta G'°$, and the Direction of Chemical Reactions

When K'_{eq} is . . .	$\Delta G'°$ is . . .	Starting with all components at 1 M, the reaction . . .
>1.0	negative	proceeds forward
1.0	zero	is at equilibrium
<1.0	positive	proceeds in reverse

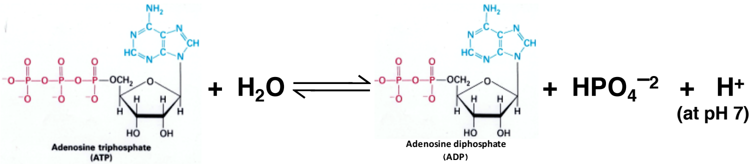
But, what is the actual $\Delta G'$ of ATP Hydrolysis IN THE CELL?

TABLE 13-2 Relationship between Equilibrium Constants and Standard Free-Energy Changes of Chemical Reactions

	$\Delta G'°$	
K'_{eq}	(kJ/mol)	(kcal/mol)[a]
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

[a] Although joules and kilojoules are the standard units of energy and are used throughout this text, biochemists and nutritionists sometimes express $\Delta G'°$ values in kilocalories per mole. We have therefore included values in both kilojoules and kilocalories in this table and in Tables 13-4 and 13-6. To convert kilojoules to kilocalories, divide the number of kilojoules by 4.184.

Metabolism



Go back the original equation: $\Delta G' = \Delta G°' + RT\ln\frac{[C]^c[D]^d}{[A]^a[B]^b}$

$$\Delta G = \Delta G°' + RT\ln\frac{[\text{MgADP}^-]\cdot[\text{P}_\text{i}]}{[\text{MgATP}^{2-}]}$$

$$\Delta G' = \Delta G°' + RT\ln\boxed{\frac{[\text{ADP}^{-3}]\,[\text{HPO}_4^{-2}]}{[\text{ATP}^{-4}]}}$$

In RBCs = 1.5×10^{-4} M

At 25 °C: $\Delta G' = \Delta G°' + 0.59\ln\frac{[\text{ADP}^{-3}]\,[\text{HPO}_4^{-2}]}{[\text{ATP}^{-4}]}$

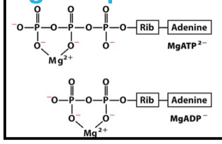
$$\Delta G' = \Delta G°' + 1.36\log(1.5 \times 10^{-4})$$

$$\Delta G' = (-7.3) + (-5.2)$$

$$\Delta G' = -12.5\ \text{kcal/mole}$$

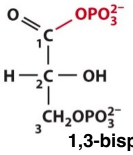
WOW!

$\Delta G°'$ of ATP Hydrolysis is Mg^{++} Dependent



Other than concentrations, what makes the $\Delta G'$ of ATP so high?

Metabolism



1,3-bisphosphoglycerate

$\Delta G^{\circ\prime} = -12$ kcal/mole

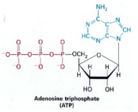
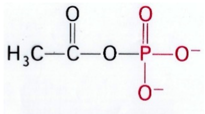
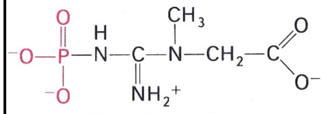


TABLE 13-4 Standard Free-Energy Changes of Some Chemical Reactions	$\Delta G'^\circ$	
Reaction type	**(kJ/mol)**	**(kcal/mol)**
Hydrolysis reactions		
Acid anhydrides		
Acetic anhydride + $H_2O \rightarrow$ 2 acetate	–91.1	–21.8
ATP + $H_2O \rightarrow$ ADP + P_i	–30.5	–7.3
ATP + $H_2O \rightarrow$ AMP + PP_i	–45.6	–10.9
$PP_i + H_2O \rightarrow 2P_i$	–19.2	–4.6
UDP-glucose + $H_2O \rightarrow$ UMP + glucose 1-phosphate	–43.0	–10.3
Esters		
Ethyl acetate + $H_2O \rightarrow$ ethanol + acetate	–19.6	–4.7
Glucose 6-phosphate + $H_2O \rightarrow$ glucose + P_i	–13.8	–3.3
Amides and peptides		
Glutamine + $H_2O \rightarrow$ glutamate 1 NH_4^+	–14.2	–3.4
Glycylglycine + $H_2O \rightarrow$ 2 glycine	–9.2	–2.2
Glycosides		
Maltose + $H_2O \rightarrow$ 2 glucose	–15.5	–3.7
Lactose + $H_2O \rightarrow$ glucose + galactose	–15.9	–3.8
Rearrangements		
Glucose 1-phosphate $\rightarrow$ glucose 6-phosphate	–7.3	–1.7
Fructose 6-phosphate $\rightarrow$ glucose 6-phosphate	–1.7	–0.4
Elimination of water		
Malate $\rightarrow$ fumarate + H_2O	3.1	0.8
Oxidations with molecular oxygen		
Glucose + $6O_2 \rightarrow 6CO_2 + 6H_2O$	–2,840	–686
Palmitate + $23O_2 \rightarrow 16CO_2 + 16H_2O$	–9,770	–2,338



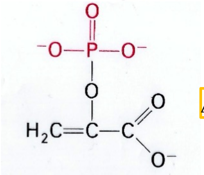
Acetyl phosphate

$\Delta G^{\circ\prime} = -11.8$ kcal/mole



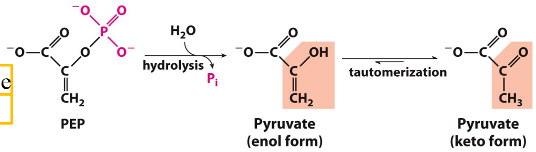
Phosphocreatine

$\Delta G^{\circ\prime} = -10.3$ kcal/mole



Phosphoenolpyruvate
(PEP)

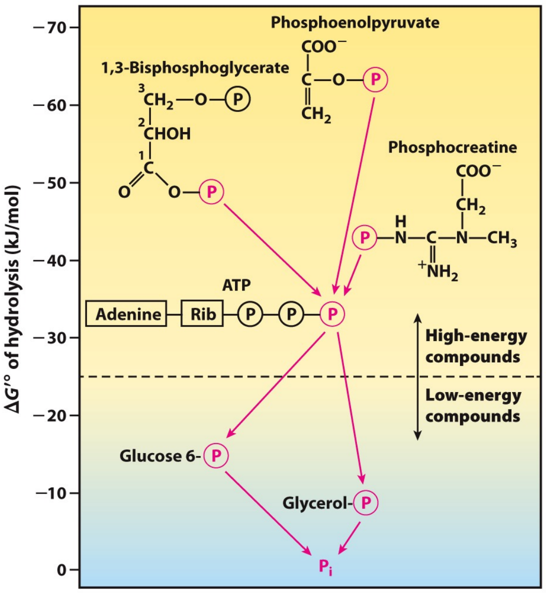
$\Delta G^{\circ\prime} = -14.8$ kcal/mole
$= -61.9$ kJ/mole



Notice:
1) anhydride or mixed anhydride bonds
2) products have more resonance forms

$$PEP^{3-} + H_2O \longrightarrow \text{pyruvate}^- + HPO_4^{2-}$$
$$\Delta G'^\circ = -61.9 \text{ kJ/mol}$$

Metabolism



Phosphates: Ranking by the *Standard* Free Energy of Hydrolysis

Phosphate can be transferred from compounds with higher $\Delta G^{\circ\prime}$ to those with lower $\Delta G^{\circ\prime}$.

Reactions such as:

PEP + ADP => Pyruvate + ATP

are favorable and can be used to synthesize ATP.

Metabolism

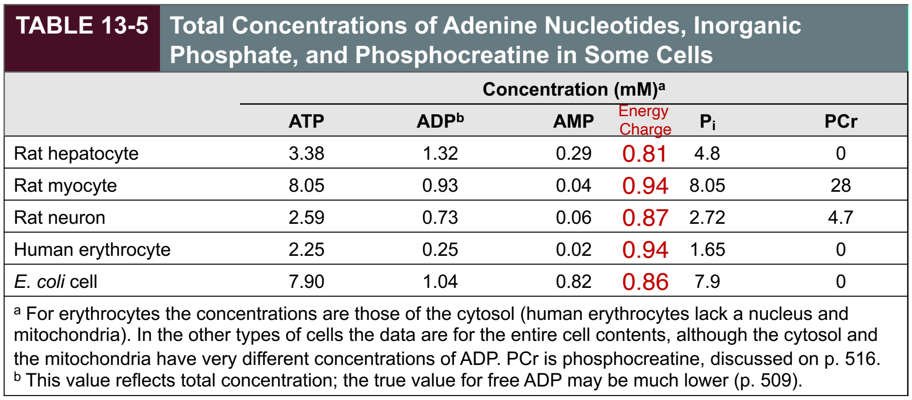


TABLE 13-5 **Total Concentrations of Adenine Nucleotides, Inorganic Phosphate, and Phosphocreatine in Some Cells**

	Concentration (mM)[a]					
	ATP	**ADP[b]**	**AMP**	Energy Charge	**P_i**	**PCr**
Rat hepatocyte	3.38	1.32	0.29	0.81	4.8	0
Rat myocyte	8.05	0.93	0.04	0.94	8.05	28
Rat neuron	2.59	0.73	0.06	0.87	2.72	4.7
Human erythrocyte	2.25	0.25	0.02	0.94	1.65	0
E. coli cell	7.90	1.04	0.82	0.86	7.9	0

[a] For erythrocytes the concentrations are those of the cytosol (human erythrocytes lack a nucleus and mitochondria). In the other types of cells the data are for the entire cell contents, although the cytosol and the mitochondria have very different concentrations of ADP. PCr is phosphocreatine, discussed on p. 516.

[b] This value reflects total concentration; the true value for free ADP may be much lower (p. 509).

Cellular ATP concentration is usually far above the equilibrium concentration, making ATP a very potent source of chemical energy.

Energy Coupling

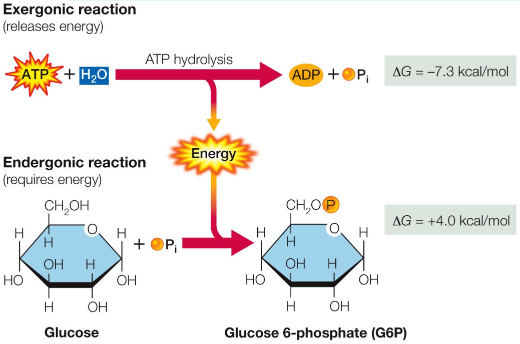
Recall that metabolism must deal with both
Favorable and Unfavorable Reactions

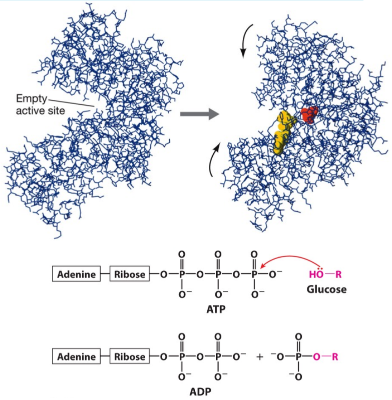
Biochemistry couples exergonic with endergonic reactions
to ensure organisms continue to grow and divide.

Energy Coupling

- Question is how do cells use these “high-energy compounds” to ensure that thermodynamically unfavorable reactions can become favorable?
- Chemical coupling of exergonic and endergonic reactions allows otherwise unfavorable reactions.
- TWO basic mechanisms:
  - e.g., The hydrolysis of the “high-energy” molecule (ATP), which is exergonic, is coupled with an otherwise endergonic reaction during an enzyme mechanism.
  - e.g., a series of otherwise endergonic reactions during a metabolic pathway are linked in the reaction series to an overwhelming exergonic reaction (all catalyzed by enzymes).

Energy Coupling

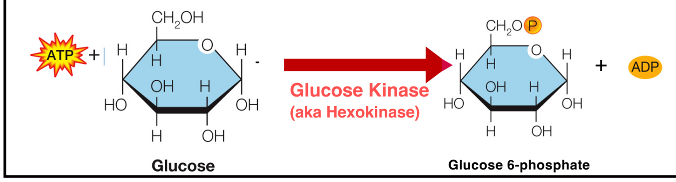




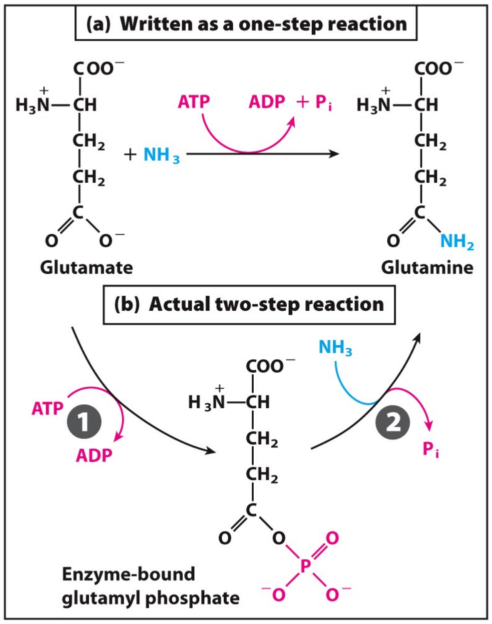
Net ΔG = –3.3 kcal/mol

Enzymatic reaction

These two reactions take place at the same place (active site)
Replace the H–O–H with H–O–R (glucose)



Energy Coupling



Two-Step Group Transfers Facilitate ATP-Dependent Reactions

Energy Coupling

Standard free-energy changes are additive:

(1) $A \rightleftarrows B \quad \Delta G_1$

(2) $B \rightleftarrows C \quad \Delta G_2$

Sum: $A \rightleftarrows C \quad \Delta G_1 + \Delta G_2$

Coupling exergonic and endergonic reactions:

$$S \rightarrow P \rightarrow Q \rightarrow R \rightarrow Z$$

$S \rightleftarrows P$ ΔG = +12 kcal/mole

$P \rightleftarrows Q$ ΔG = +1 kcal/mole

$Q \rightleftarrows R$ ΔG = -4 kcal/mole

$R \rightleftarrows Z$ ΔG = -14 kcal/mole

Net: $S \rightleftarrows Z$ ΔG = -5 kcal/mole

Chemical Reactivity

First lets quickly review bond cleavage and nucleophilicity in terms of biochemical instances

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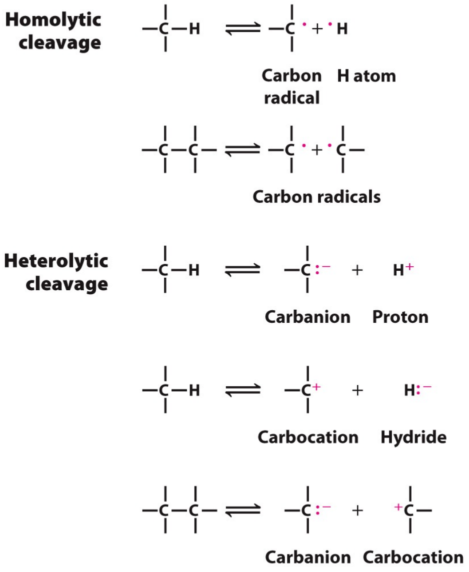
Chemical Reactivity

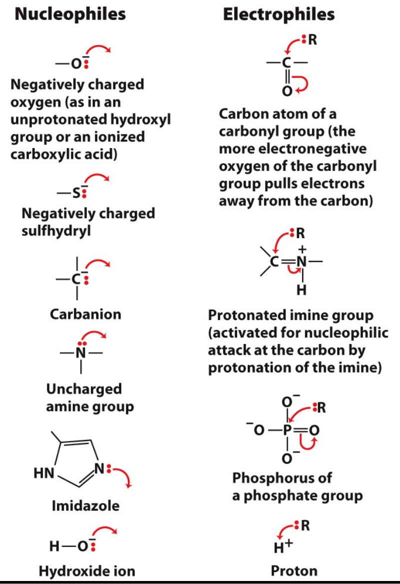
Chemistry at Carbon: Homolytic versus Heterolytic Cleavage



- Covalent bonds can be broken in two ways.
- Homolytic cleavage is rare in biochemistry but makes up some of the most interesting reactions.
- Heterolytic cleavage is the most common, but the products are highly unstable, dictating the chemistry that occurs….. mostly carbanion chemistry

Chemical Reactivity

Nucleophiles and Electrophiles in Biochemistry



Definition: *-phile* (suffix, Latin): to love, or demonstrate fondess towards

- Nucleophiles love nuclei. The nuclei of an atom contains positively charged particles (protons), so nucleophiles love to react with positive charges and are electron rich.
- Electrophiles love electrons. Electrons are negatively charged, so electrophiles must be positively charged.

Chemical Reactivity

Most biochemical reactions fall within only a few categories:

- •Cleavage and formation of C–C bonds
- •Cleavage and formation of polar covalent bonds (Addition-Elimination Reactions)
  - –nucleophilic substitution mechanism
  - –addition–elimination mechanism
    - o hydrolysis and condensation reactions
    - o Eliminations (without cleavage)
- •Internal rearrangements
- •Group transfers (H^+, CH_3, PO_3^{2-}, NH_3^+)
- •Oxidations-reductions (e^- transfers)