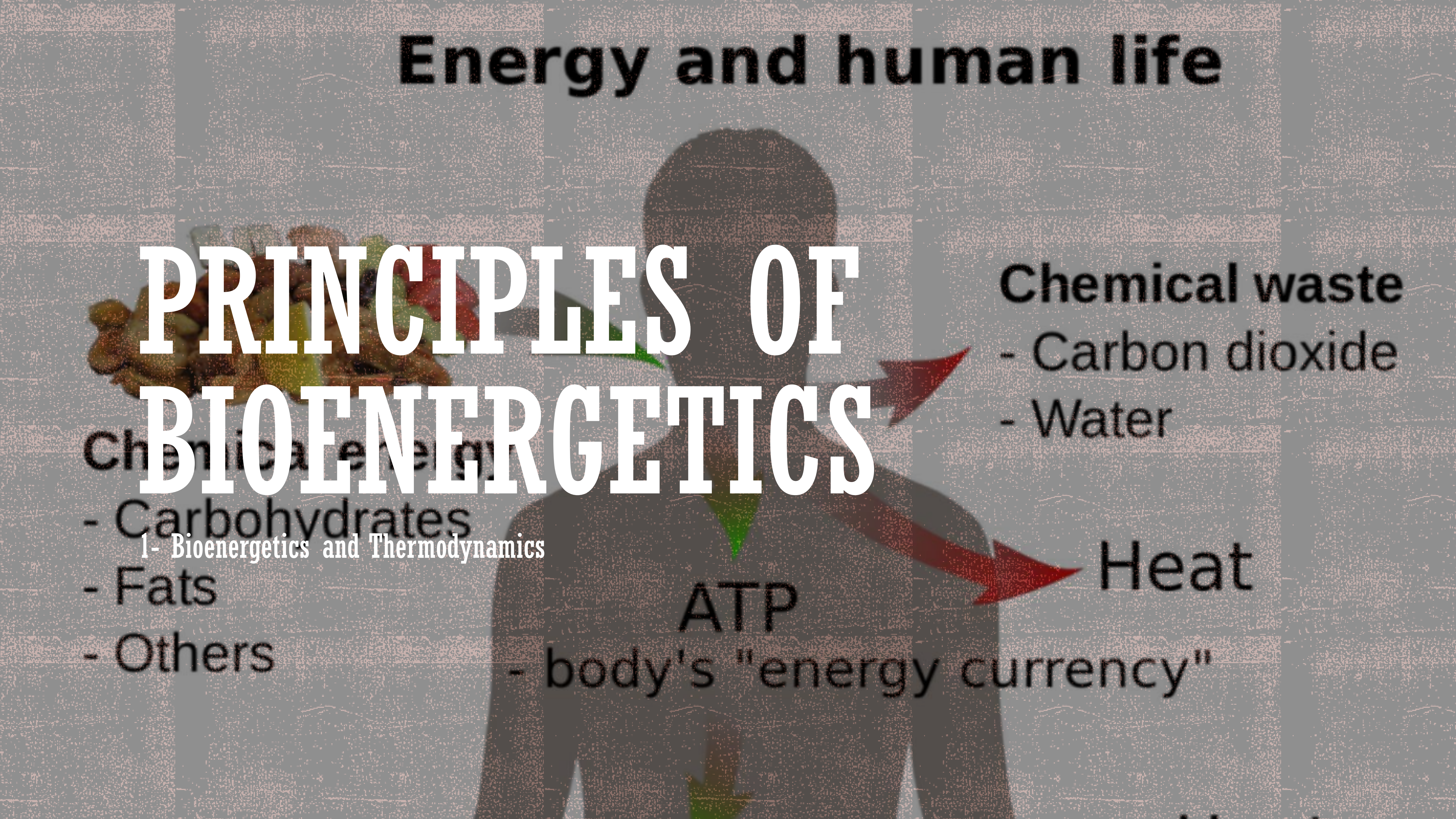
PRINCIPLES OF BIOENERGETICS

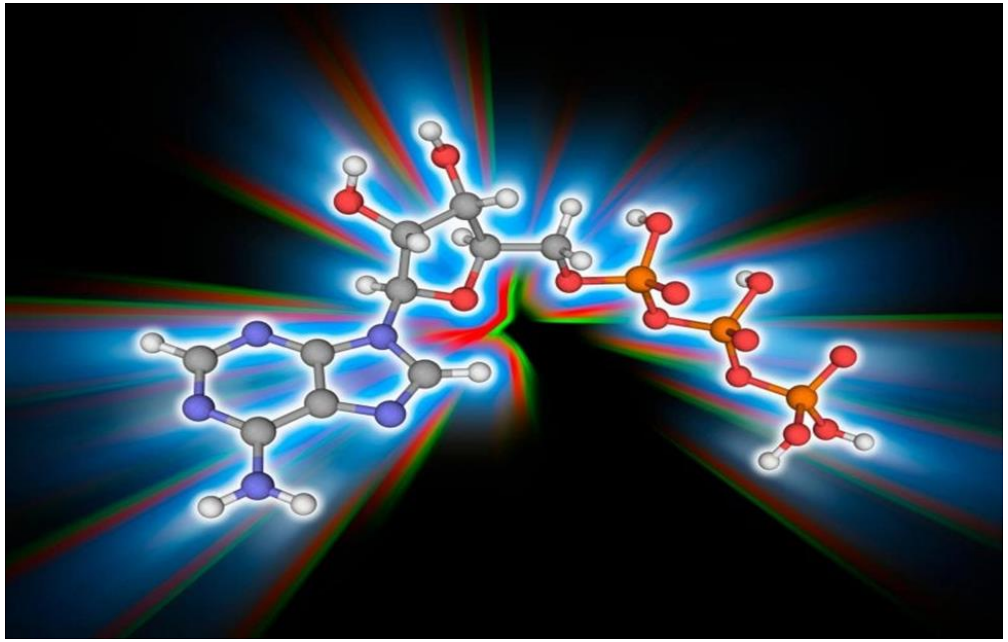
1- Bioenergetics and Thermodynamics

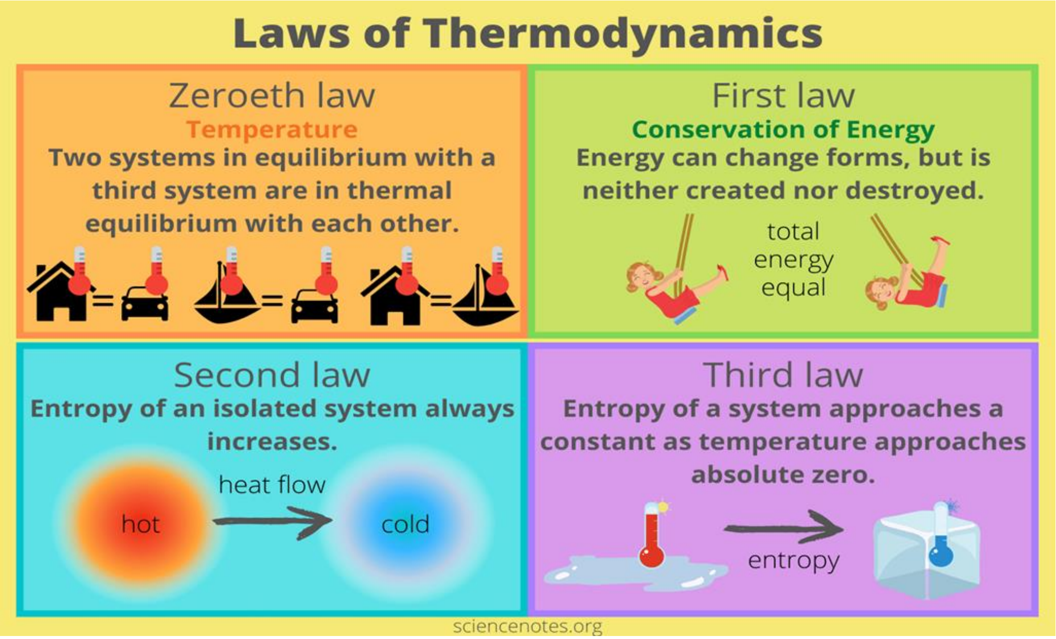


DEFINITION

- The **quantitative study of the energy transductions** that occur in living cells and of the nature and function of the chemical processes underlying these transductions.

LECTURE 1: THE LAWS OF ENERGY AND CELLULAR CURRENCY





THE CLINICAL STAGE: A CASE OF LETHARGY

- Clinical Presentation
  - A 2-month-old infant presents with lethargy, poor feeding, and hypoglycemia indicating a metabolic crisis.
- Laboratory Findings
  - Initial labs reveal low blood glucose and mild metabolic acidosis, signaling disrupted metabolism.
- Biochemical Diagnostic Question
  - The clinical challenge is differentiating fuel deficiency from impaired energy utilization in the patient.
- Importance of Bioenergetics
  - Understanding bioenergetics is critical for diagnosing and managing metabolic crises in pediatric patients.

BIOLOGICAL ENERGY TRANSFORMATIONS OBEY THE LAWS OF THERMODYNAMICS

The 1st law (conservation of energy): for any change, the total amount of energy in the universe remains constant;

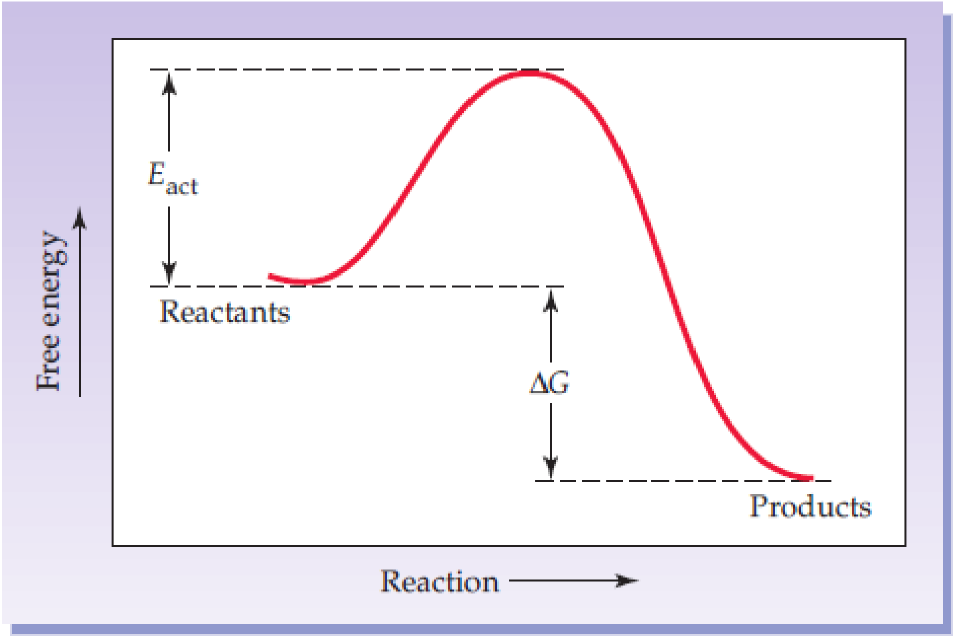
The 2nd law of thermodynamics: the universe always tends toward increasing disorder (or); in all natural processes, the entropy of the universe increases.

Signs for spontaneous systems…

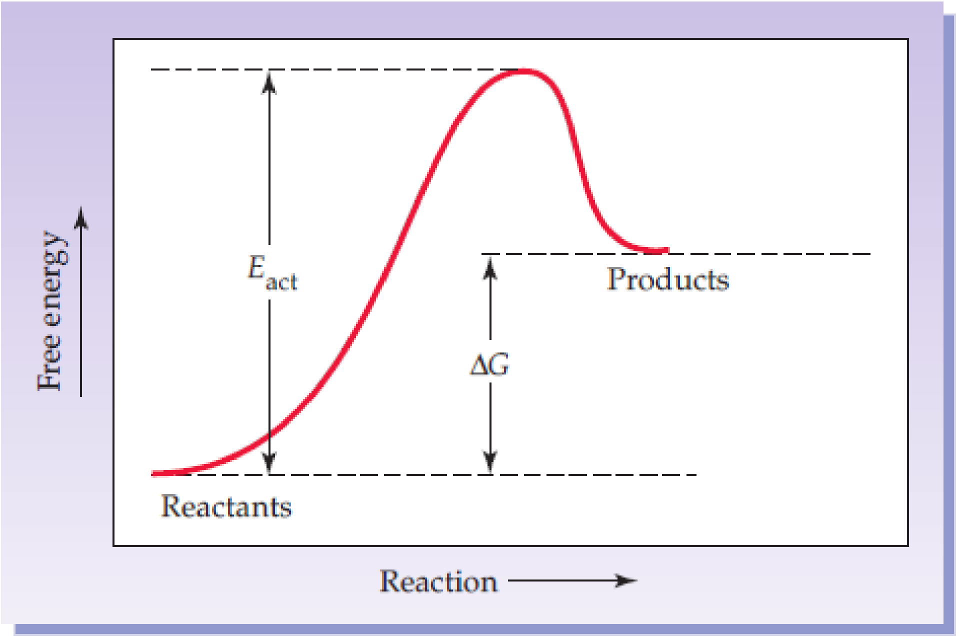
$$\Delta G = \Delta H - T\Delta S$$

WHY DO CHEMICAL REACTIONS OCCUR?

- The concept of activation energy
- ΔG and ΔG^0



(a) An exergonic reaction



(b) An endergonic reaction

ΔG IS A STATE FUNCTION?!

- **ΔG is not affected by the mechanism of the reaction**
- $A \rightarrow B \rightarrow C$

$$\Delta G_{A\rightarrow B} = \cancel{GB} - GA$$
$$\Delta G_{B\rightarrow C} = GC - \cancel{GB}$$
$$\overline{\qquad GC - GA = \Delta G_{A\rightarrow C}}$$

- Combustion of glucose in calorimeter

Glucose + $O_2 \rightarrow CO_2 + H_2O$ $\quad \Delta G = -\ 680$ kcal/mol

In the cell

Glucose $\rightarrow\rightarrow\rightarrow\rightarrow\rightarrow CO_2 + H_2O$ $\quad \Delta G = -\ 680$ kcal/mol

ΔG IS AFFECTED BY CONCENTRATION

$A \longrightarrow B$ $\Delta G = - - -$

$A \rightleftharpoons$

$A \rightleftharpoons$

$A \rightleftharpoons$

HOW ΔG AND ΔG^0 RELATE?

- Concentrations of reactants and products = 1 mole/L

- $\Delta G = \Delta G^0 + RT \ln \dfrac{[\text{Products}]}{[\text{Reactants}]}$

- $\Delta G = \Delta G^0 + RT \; 2.3 \log \dfrac{[\text{Products}]}{[\text{Reactants}]}$

THE STANDARD FREE-ENERGY CHANGE IS DIRECTLY RELATED TO THE EQUILIBRIUM CONSTANT (Keq)

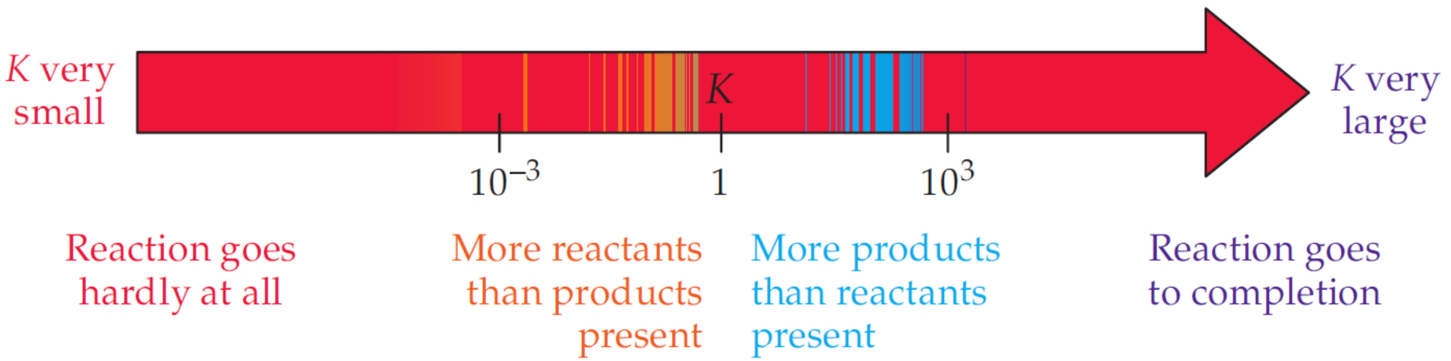
Equilibrium is reached at the end!

At equilibrium: concentration are fixed; rates are exactly equal

The concentrations define the equilibrium constant

$$aA + bB \rightleftharpoons cC + dD$$

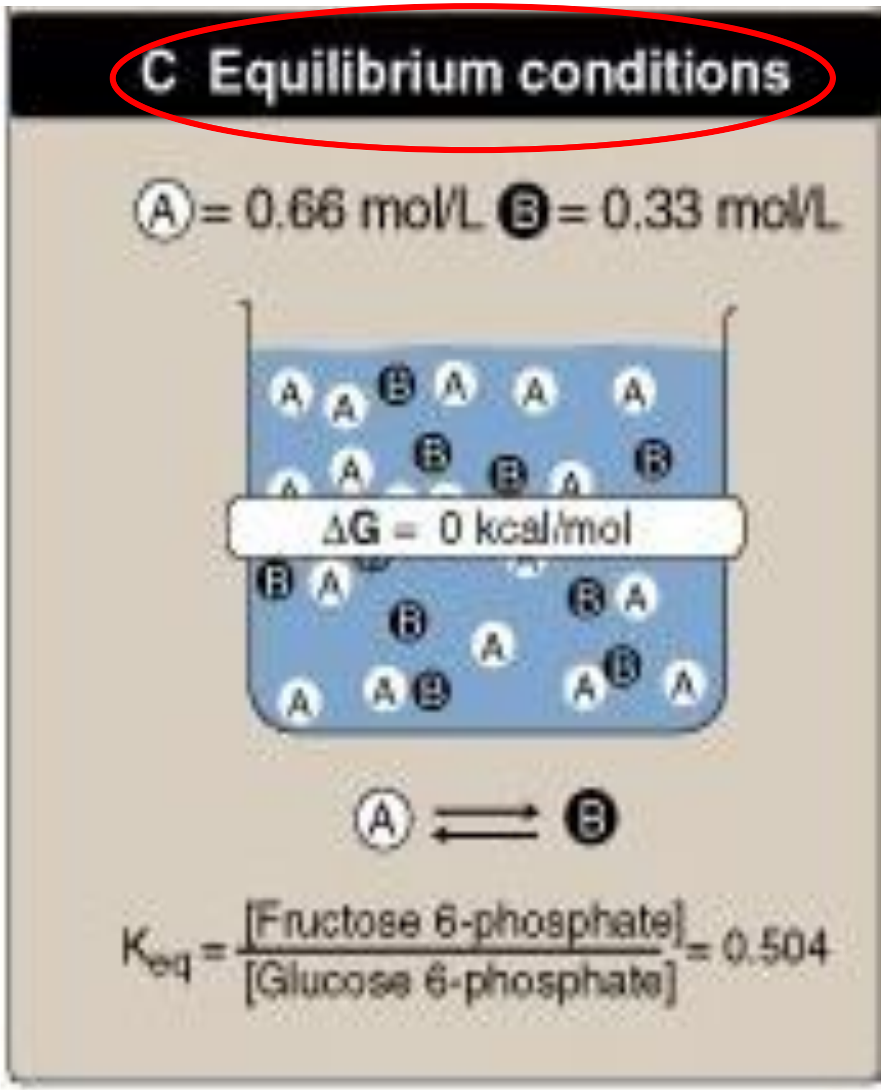
$$K_{eq} = \frac{[C]^c[D]^d}{[A]^a[B]^b}$$



$$\Delta G = \Delta G^{\circ} + RT \ln \frac{[Products]}{[Reactants]}$$

Glucose 6- phosphate $\rightleftharpoons$ Fructose 6- phosphate

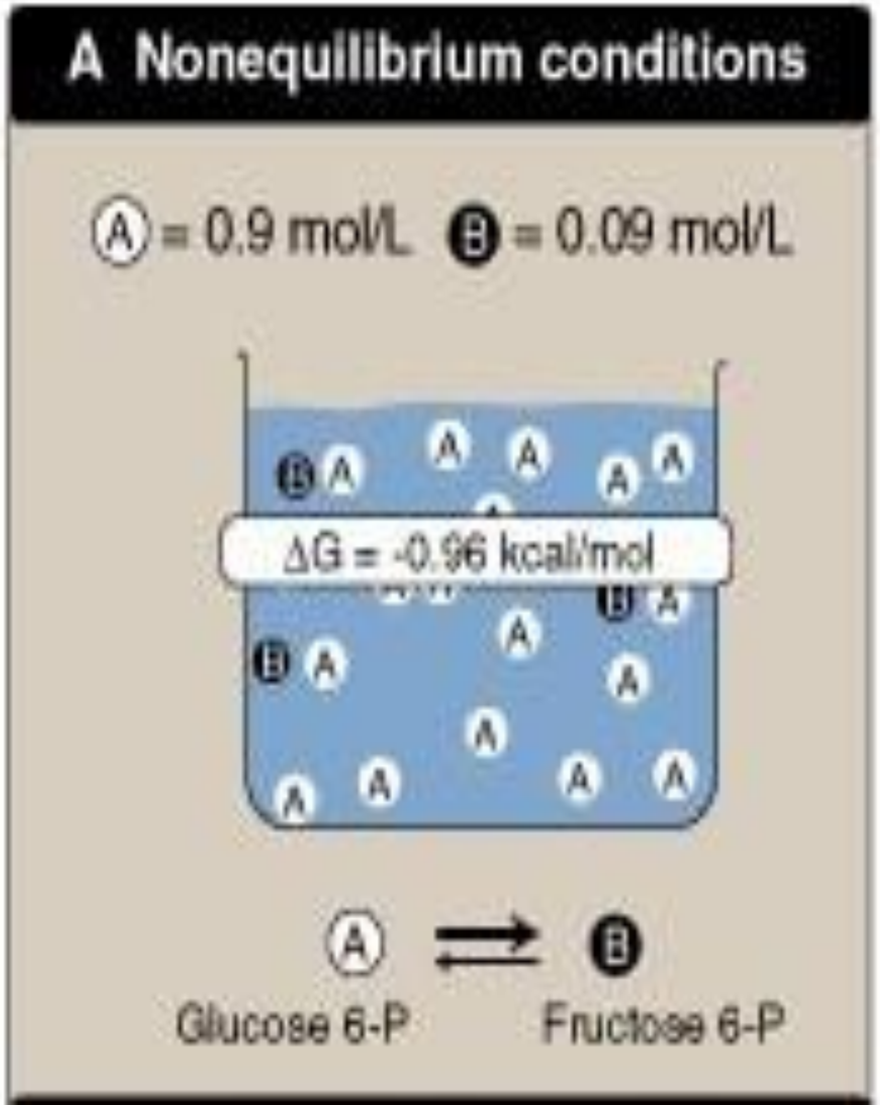
0.66 mol/L 0.33 mol/L



$\Delta G = \Delta G^{o} + RT\ 2.3 \log 0.33/\ 0.66$

$\Delta G^{o} = +\ 0.4$ kcal/mol

Glucose 6- phosphate $\rightleftharpoons$ Fructose 6- phosphate



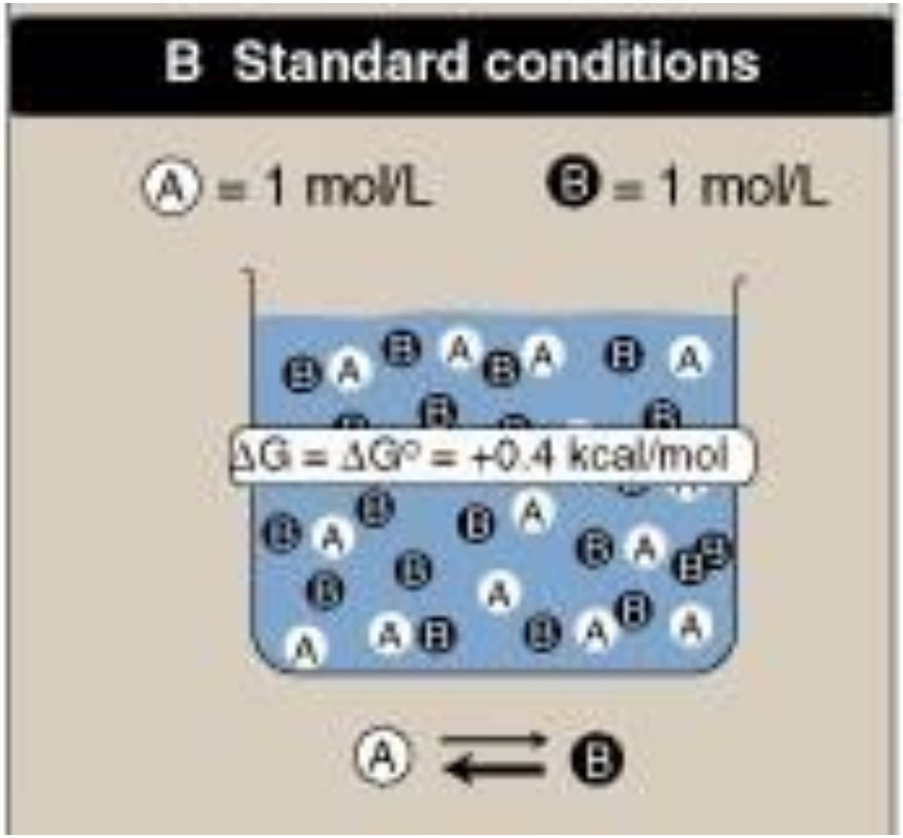
$\Delta G^o = +0.4$ kcal/mol

$\Delta G = \Delta G^o + RT\ 2.3 \log 0.09/0.9$

$\Delta G = -0.96$

Glucose 6- phosphate $\rightleftharpoons$ Fructose 6- phosphate

1 mol/L 1 mol/L



$\Delta G = \Delta G^o + RT\ 2.3 \log 1/1$

$\Delta G = \Delta G^o$

ΔG & K_{eq}

For a reaction $A + B \leftrightarrow C + D$

$$\Delta G = \Delta G^{o\prime} + RT \ln \left(\frac{[C]\,[D]}{[A]\,[B]}\right)$$

- At equilibrium, $\Delta G = 0$
- Can a reaction has a +ve ΔG^0 & still be favorable?

K'_{eq}	$\Delta G^{o\prime}$ kJ/mol	Starting with 1 M reactants & products, the reaction:
10^4	- 23	proceeds forward (spontaneous)
10^2	- 11	proceeds forward (spontaneous)
$10^0 = 1$	0	is at **equilibrium**
10^{-2}	+ 11	reverses to form “reactants”
10^{-4}	+ 23	reverses to form “reactants”

$$\Delta G = \Delta G^{o\prime} + RT \ln \left(\frac{[C]\,[D]}{[A]\,[B]}\right)$$

$$0 = \Delta G^{o\prime} + RT \ln \left(\frac{[C]\,[D]}{[A]\,[B]}\right)$$

$$\Delta G^{o\prime} = - RT\ln \left(\frac{[C]\,[D]}{[A]\,[B]}\right)$$

$$\text{defining } K'_{eq} = \left(\frac{[C]\,[D]}{[A]\,[B]}\right)$$

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

ΔG^{o} AND K_{eq}

1	0

How much change in delta G compared to changes in Keq

If $K_{eq} = 1$, then $\Delta G^{o} = 0$

If $Keq > 1$, then $\Delta G^{o} < 0$

If $Keq < 1$, then $\Delta G^{o} > 0$

$$\Delta G = \Delta G^{o} + RT \ln \frac{[\text{Products}]}{[\text{Reactants}]}$$

ΔG & K_{eq}

- ΔG is additive for multiple subsequent reactions
- K_{eq} is multiplicative for subsequent reactions

$$\text{Glucose} + P_i \longrightarrow \text{glucose 6-phosphate} + H_2O \qquad \Delta G'^{\circ} = 13.8 \text{ kJ/mol}$$

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

$$\begin{array}{ll} (1) & \text{Glucose} + P_i \longrightarrow \text{glucose 6-phosphate} + H_2O \\ (2) & \text{ATP} + H_2O \longrightarrow \text{ADP} + P_i \\ \hline \text{Sum:} & \text{ATP} + \text{glucose} \longrightarrow \text{ADP} + \text{glucose 6-phosphate} \end{array}$$

$$\Delta G'^{\circ} = 13.8 \text{ kJ/mol} + (-30.5 \text{ kJ/mol}) = -16.7 \text{ kJ/mol}$$

$$K'_{eq_1} = \frac{[\text{glucose 6-phosphate}]}{[\text{glucose}][P_i]} = 3.9 \times 10^{-3}\ \text{M}^{-1}$$

$$K'_{eq_2} = \frac{[\text{ADP}][P_i]}{[\text{ATP}]} = 2.0 \times 10^{5}\ \text{M}$$

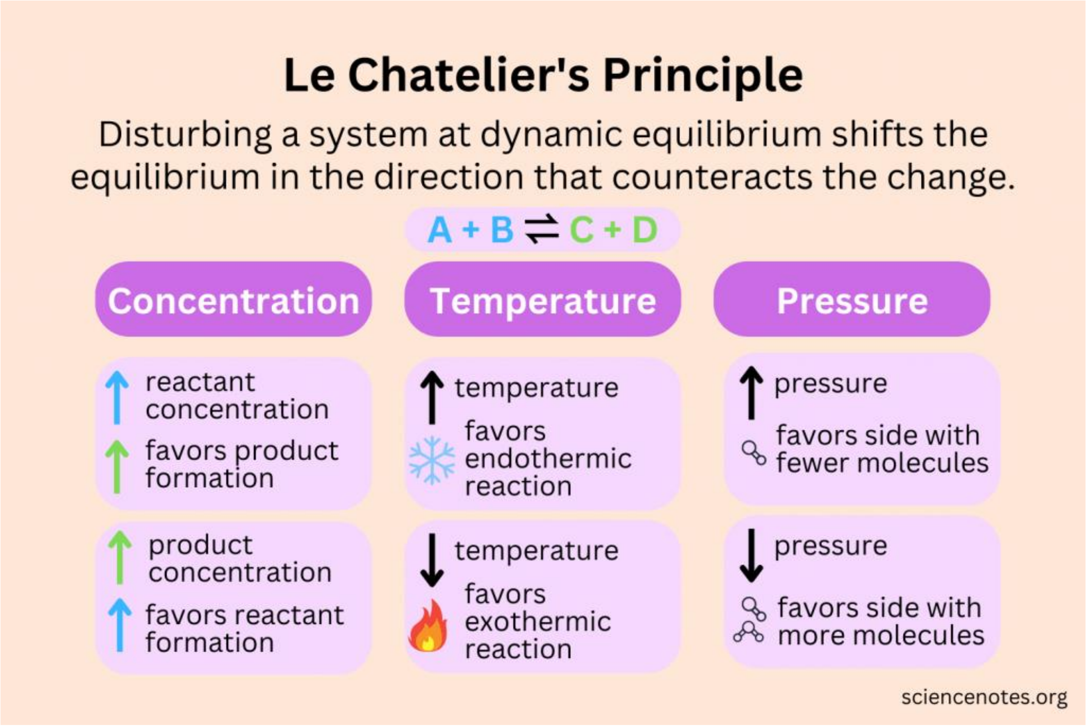
$$K'_{eq_3} = \frac{[\text{glucose 6-phosphate}][\text{ADP}][P_i]}{[\text{glucose}][P_i][\text{ATP}]}$$

$$= (K'_{eq_1})(K'_{eq_2}) = (3.9 \times 10^{-3}\ \text{M}^{-1})\ (2.0 \times 10^{5}\ \text{M})$$

$$= 7.8 \times 10^{2}$$

THE EFFECT OF CHANGING CONDITIONS ON EQUILIBRIA

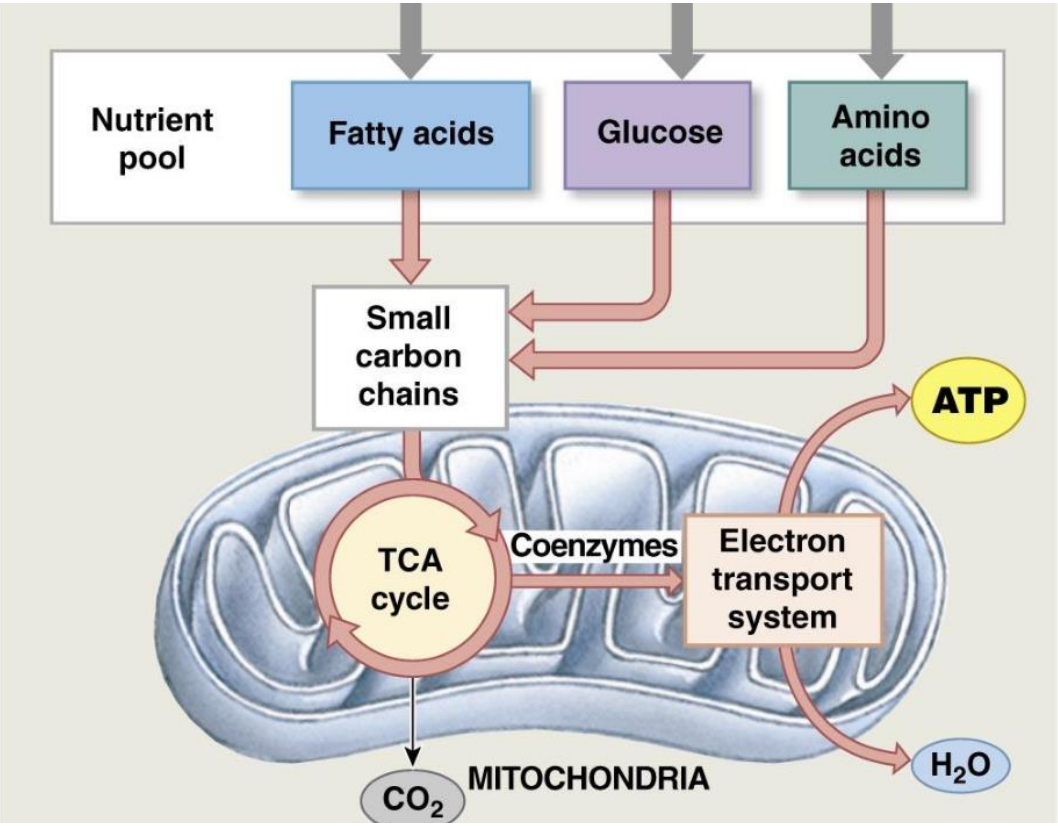
- When a stress is applied to a system at equilibrium, the equilibrium shifts to relieve the stress
- Effect of Changes in Concentration
  - What happens if a reactant/product is continuously supplied/ removed?
  - Metabolic reactions sometimes take advantage of this effect
- Effect of Changes in Temperature
  - Endothermic/exothermic are favored by increase/decrease in temperature, respectively.
- Effect of a catalyst on equilibrium



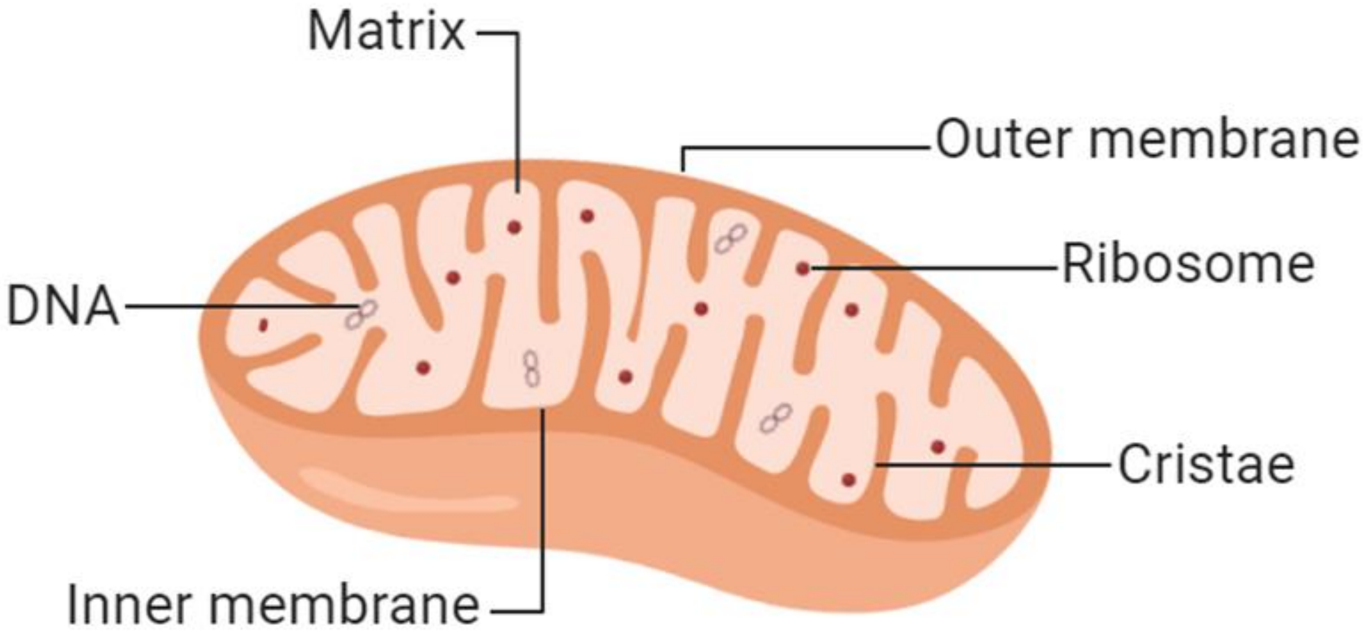
PRINCIPLES OF BIOENERGETICS

2- Phosphoryl Group Transfers and ATP

THE ENERGY FLOW



- Ingestion, digestion, & absorption
- Metabolism (Acetyl CoA)
- TCA
- Oxidative phosphorylation



THE ENERGY MACHINERY OF THE CELL

- Prokaryotic cells vs. eukaryotic cells
- The mitochondria (singular, mitochondrion) (90% of the body's energy ATP)
- The number of mitochondria is greatest in eye, brain, heart, & muscle, where the need for energy is greatest
- The ability of mitochondria to reproduce (athletes)
- Maternal inheritance

ATP

- ATP is the energy currency of the cell
- What is a high energy molecule?
- Why ATP?
  - Has an intermediate energy value, so can be coupled

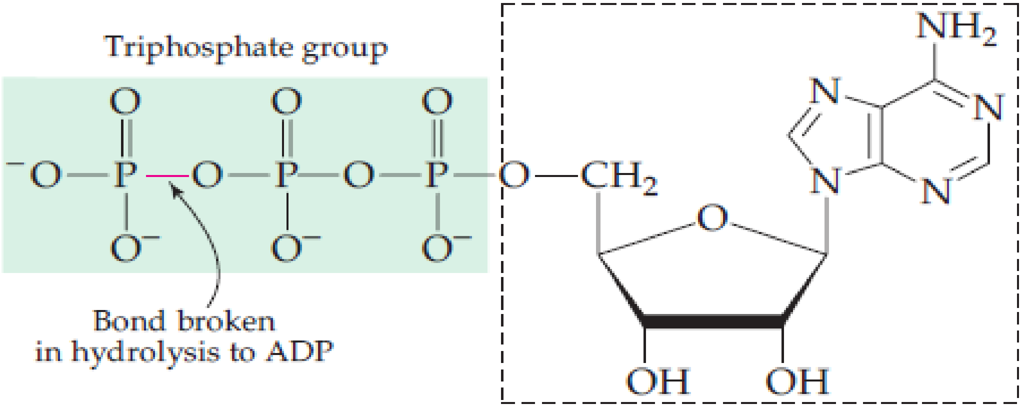
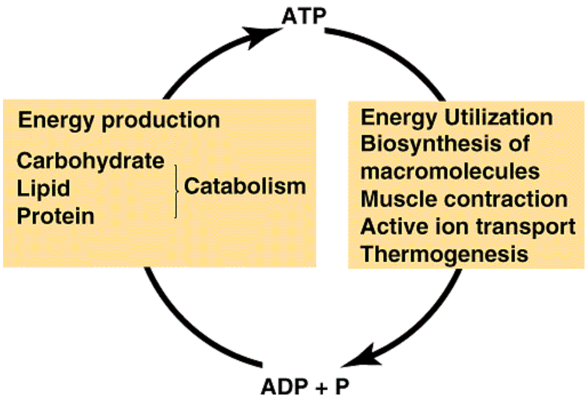


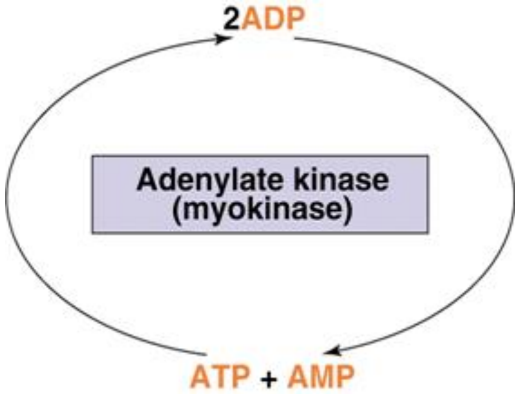
TABLE 13–6 Standard Free Energies of Hydrolysis of Some Phosphorylated Compounds and Acetyl-CoA (a Thioester)

	$\Delta G'°$ (kJ/mol)	$\Delta G'°$ (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

IS ATP A GOOD LONG-TERM ENERGY STORAGE MOLECULE?

- As food in the cells is gradually oxidized, the released energy is used to re-form the ATP so that the cell always maintains a supply of this essential molecule



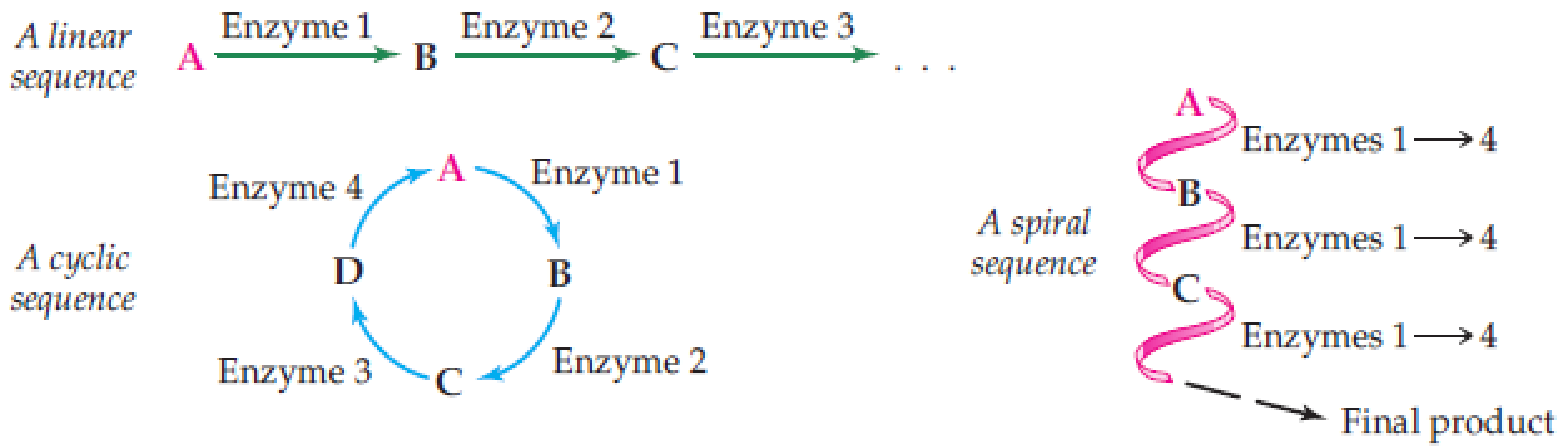


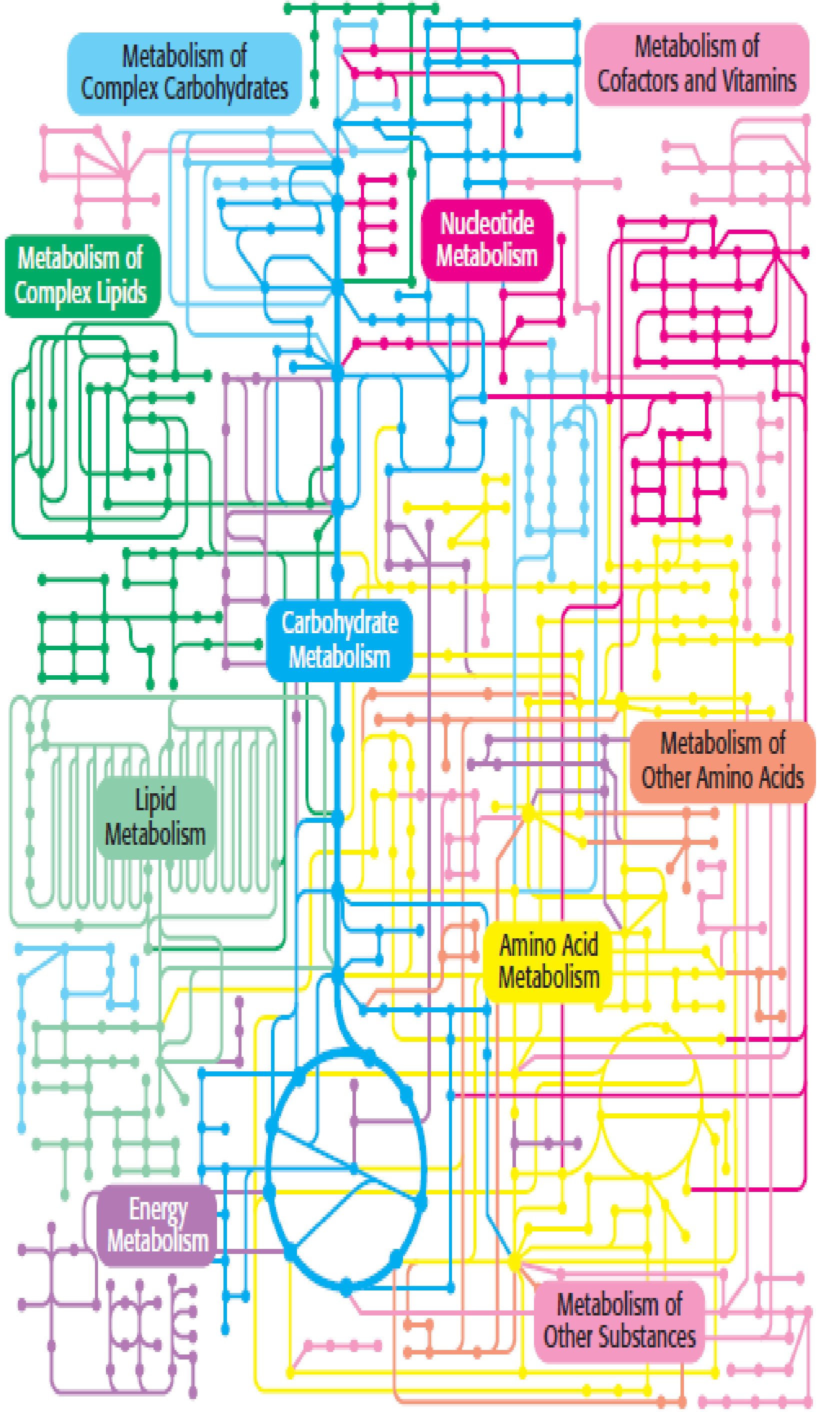
Tissue	ATP turnover (mole/day)
Brain	20.4
Heart	11.4
Kidney	17.4
Liver	21.6
Muscle	19.8
Total	90.6

90.6 * 551 (g/mole) = 49,920 g ATP

BIOCHEMICAL REACTIONS OR PATHWAYS!

- **Are <u>interdependent</u>**
- **Are subjected to thermodynamics laws**
- **Coordinated by sensitive means of communication**
- **<u>Allosteric enzymes</u> are the predominant regulators**
- **Pathways are <u>linear, cyclic or spiral</u>**





EXERGONIC REACTIONS AND PATHWAYS IN BIOCHEMISTRY

- Complex structures ➔ simple structures

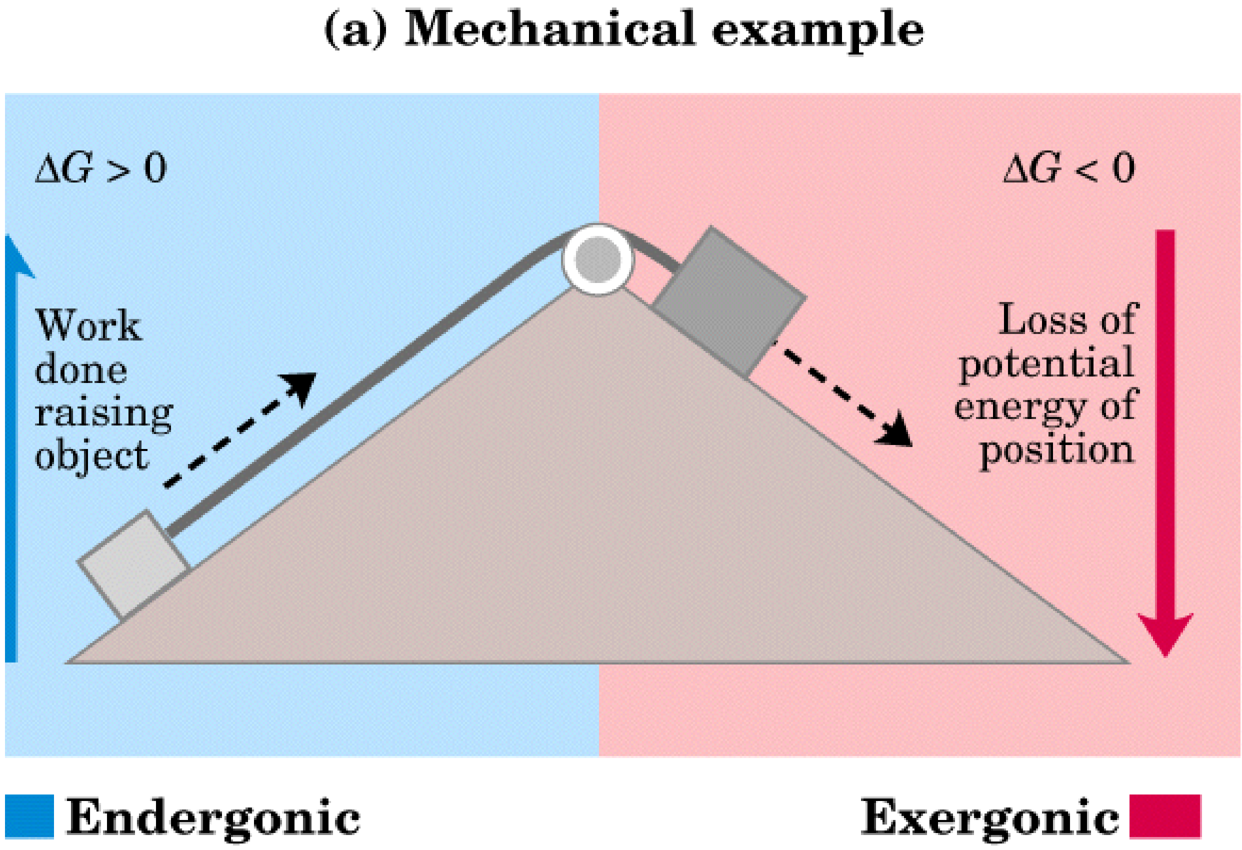
  Proteins → amino acids

  Starch → n glucose

  glucose + O_2 → CO_2 + H_2O

- More specifically
  - Hydrolysis reactions
  - Decarboxylation reactions (release of CO_2)

    pyruvate (C3) → acetyl-CoA(C2) +CO_2
  - Oxidation with O_2

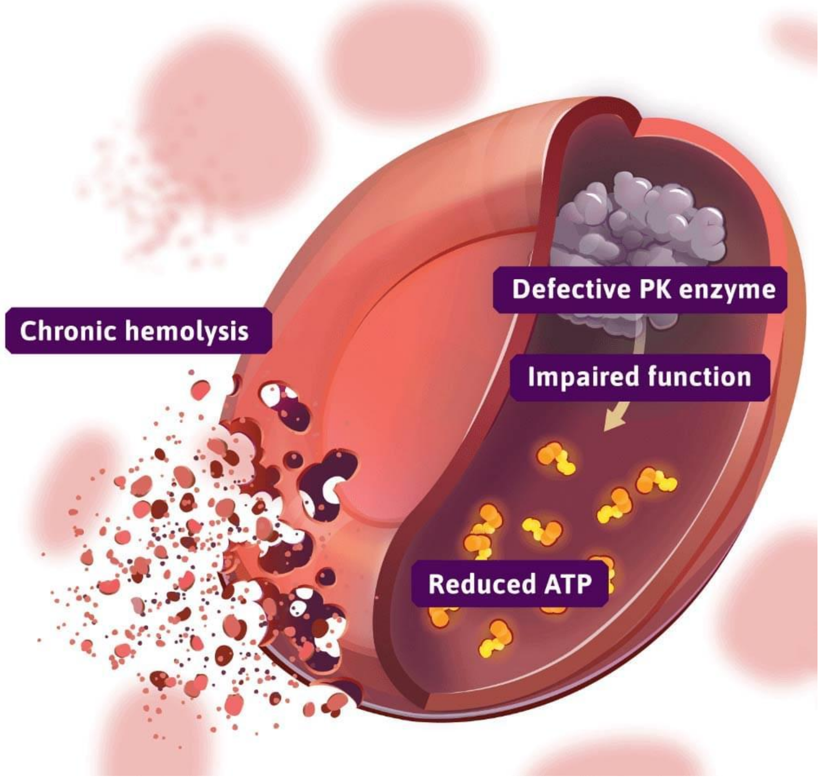


HOW DO OUR CELLS GET ENERGY FOR UNFAVORABLE BIOCHEMICAL WORK? MAKING THE IMPOSSIBLE POSSIBLE

The coupling concept - **phosphoryl transfer reactions**

CLINICAL CASE: PYRUVATE KINASE DEFICIENCY

- **Role of Pyruvate Kinase**
  - Pyruvate kinase catalyzes a crucial step in glycolysis important for ATP generation in red blood cells.
- **Impact of Deficiency**
  - Deficiency in pyruvate kinase reduces ATP production, causing red blood cell rigidity and hemolytic anemia.
- **Energy Coupling in Cells**
  - This case highlights how energy coupling via ATP is vital for maintaining cellular health and function.

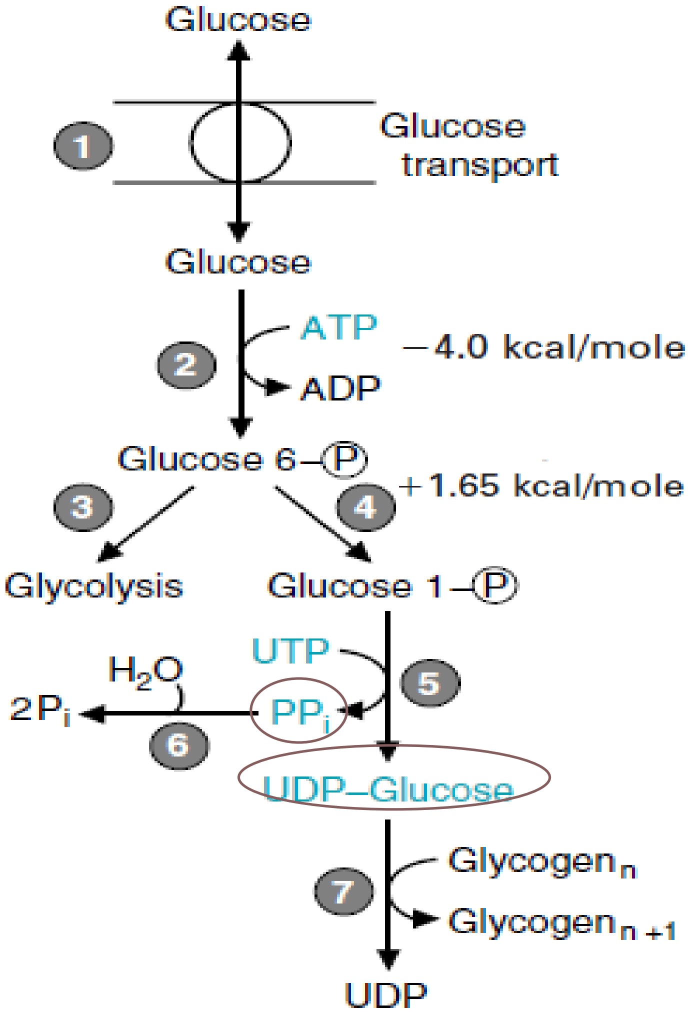


HOW DO OUR CELLS GET ENERGY FOR UNFAVORABLE BIOCHEMICAL WORK?

I. **ΔG^0 Values are additive**
   i. **Through phosphoryl transfer reactions:**
      - **Step 2 (+3.3 vs. -4 kcal/mole)**
      - **Step 2 + 4 = -2.35 kcal/mole**
      - **<u>The net value for synthesis is irrelevant to the presence or absence of enzymes</u>**

   ii. **Activated intermediates (step 4 is facilitated by steps 5&6)**

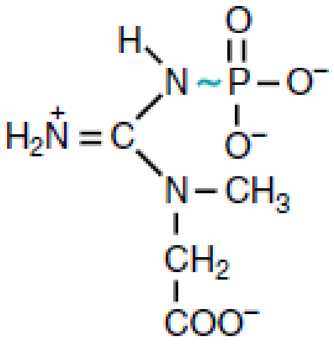
II. **ΔG Depends on Substrate and Product Concentration (step 4 has a ratio of 6/94; +1.65 kcal/mol, if 3/94; -0.4kcal/mol)**



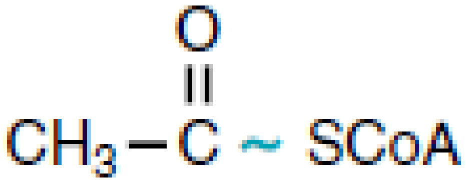
THE FREE-ENERGY CHANGE FOR ATP HYDROLYSIS IS LARGE AND NEGATIVE

	Concentration (mM)*			
	ATP	ADP†	AMP	P_i
Rat hepatocyte	3.38	1.32	0.29	4.8
Rat myocyte	8.05	0.93	0.04	8.05
Rat neuron	2.59	0.73	0.06	2.72
Human erythrocyte	2.25	0.25	0.02	1.65
E coli cell	7.90	1.04	0.82	7.9

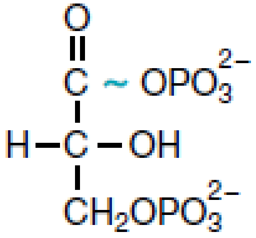
Not fixed and depends on concentrations



Creatine phosphate



Acetyl CoA



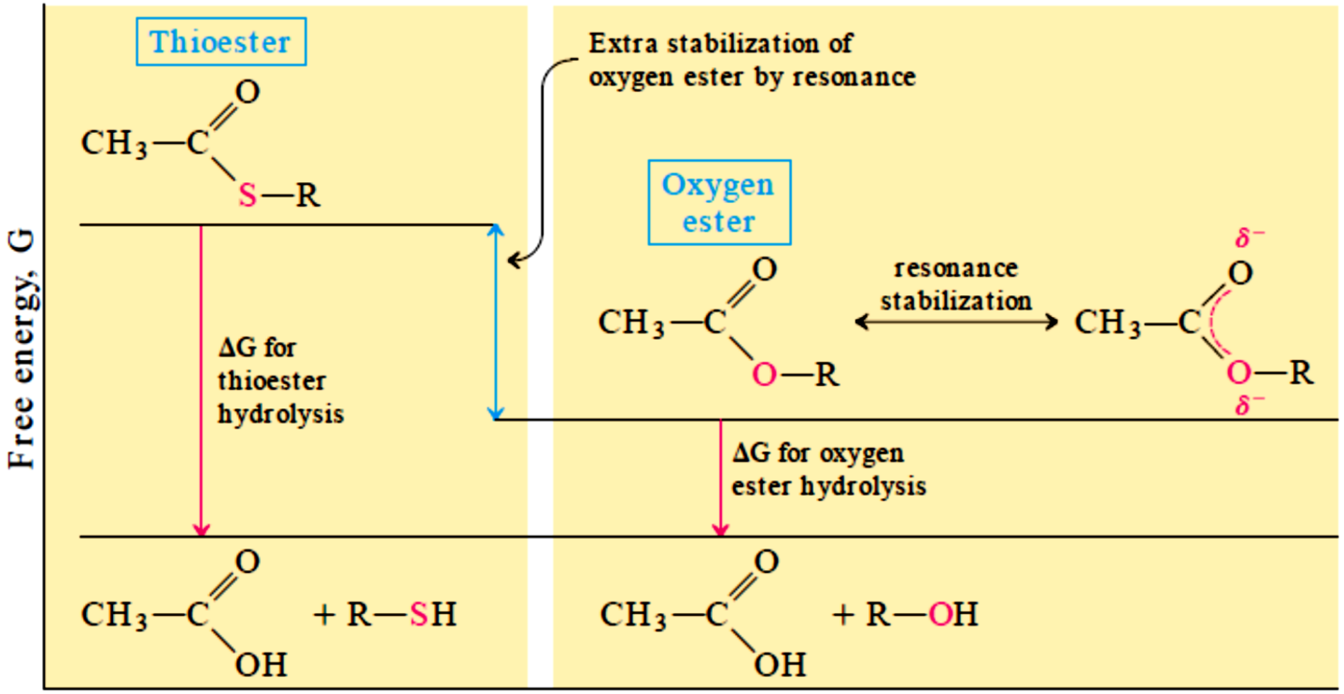
1,3–Bisphosphoglycerate

HOW DO OUR CELLS GET ENERGY FOR UNFAVORABLE BIOCHEMICAL WORK?

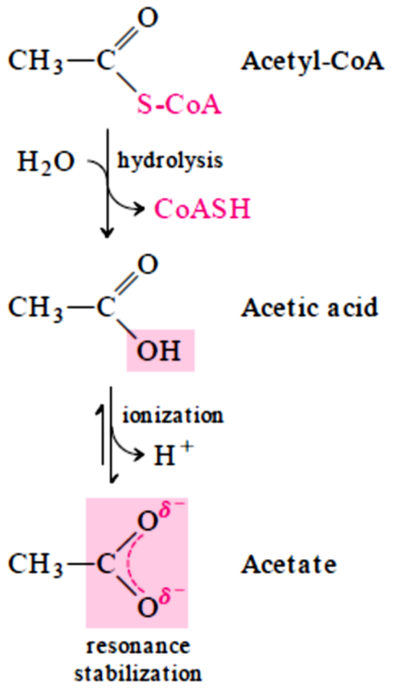
III. **Activated Intermediates other than ATP; UTP is used for combining sugars, CTP in lipid synthesis, and GTP in protein synthesis**

THE ACETYL COA AS AN EXAMPLE OF COUPLING

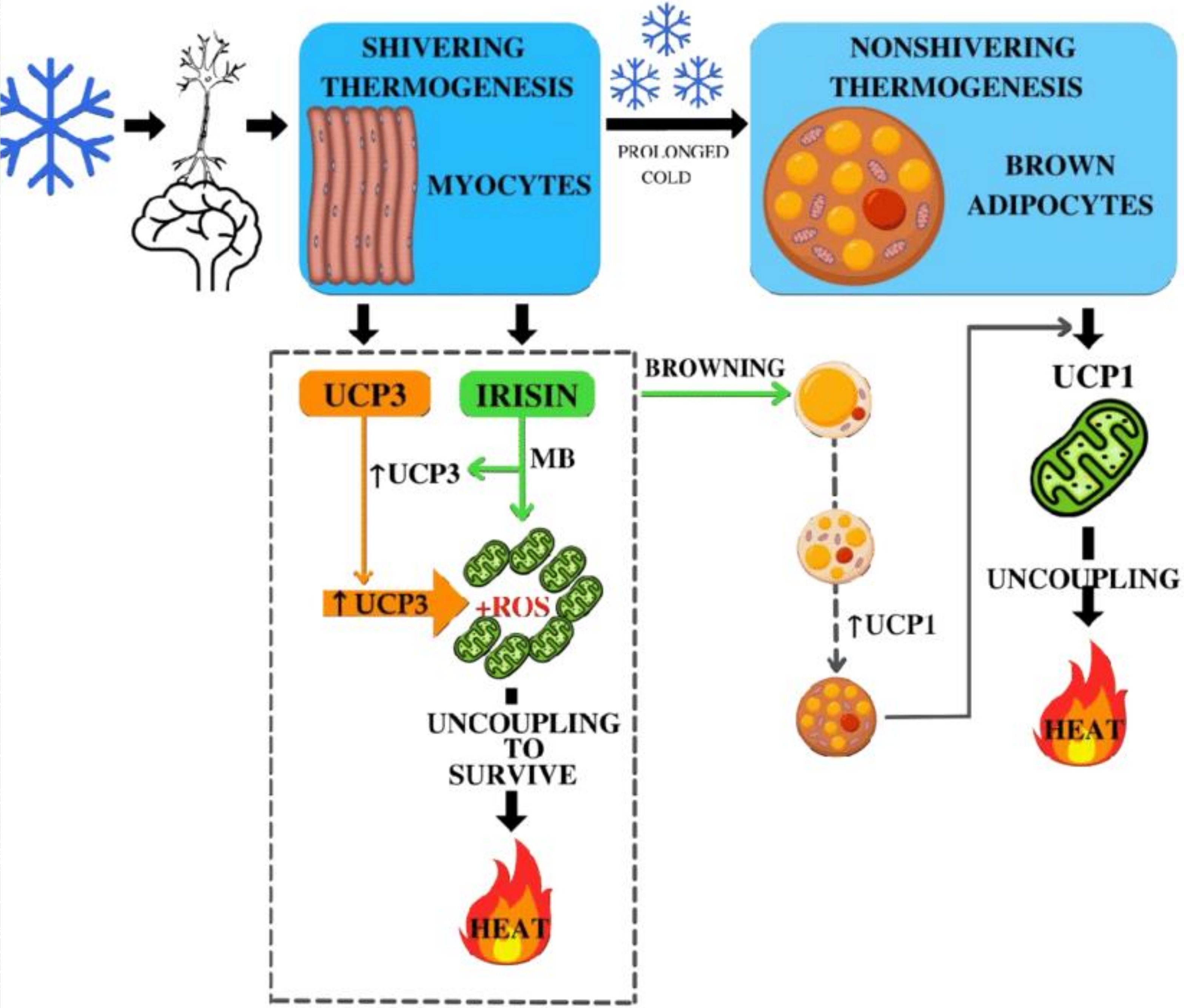
- CoA is a universal carrier (donor) of Acyl groups
- Forms a thio-ester bond with carboxyl group



- Acetyl CoA + H_2O ⟶ Acetate + CoA $\Delta G^0 = -7.5$ kcal
- Acetylcholine + H_2O ⟶ Acetate + Choline $\Delta G^0 = -3$ kcal



Acetyl-CoA + H_2O ⟶ $acetate^-$ + CoA + H^+
$\Delta G'^\circ = -31.4$ kJ/mol



THERMOGENESIS

- The first law of thermodynamics
- Heat production is a natural consequence of "burning fuels"
- Thermogenesis refers to energy expended for generating heat
- Shivering thermogenesis
- Non-shivering thermogenesis

PRINCIPLES OF BIOENERGETICS

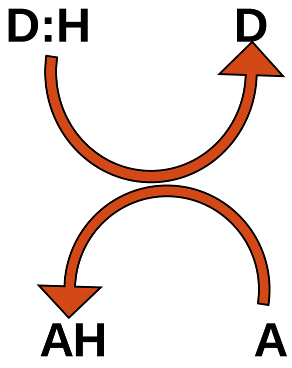
3- Biological Oxidation-Reduction Reactions

(Redox)

OXIDATION-REDUCTION REACTIONS (REDOX)

- **Oxidation:**
    - **Gain of Oxygen**
    - **Loss of Hydrogen**
    - **Loss of electrons**
- **Reduction**:
    - Gain of Hydrogen
    - Gain of electron
    - Loss of Oxygen

- E (redox Potential): it is a POTENTIAL ENERGY that measures the tendency of oxidant/reductant to gain/lose electrons, to become reduced/oxidized
- Electrons move from compounds with lower reduction potential (more negative) to compounds with higher reduction potential (more positive)
- Oxidation and reduction must occur simultaneously



REDUCTION POTENTIAL

- A-P + B $\longrightarrow$ A + B-P

Type of reaction?

What determine the direction of the reaction?

- $A^{++} + B^{++} \longrightarrow A^{+} + B^{+++}$

Type of reaction

What determine the direction of the reaction?

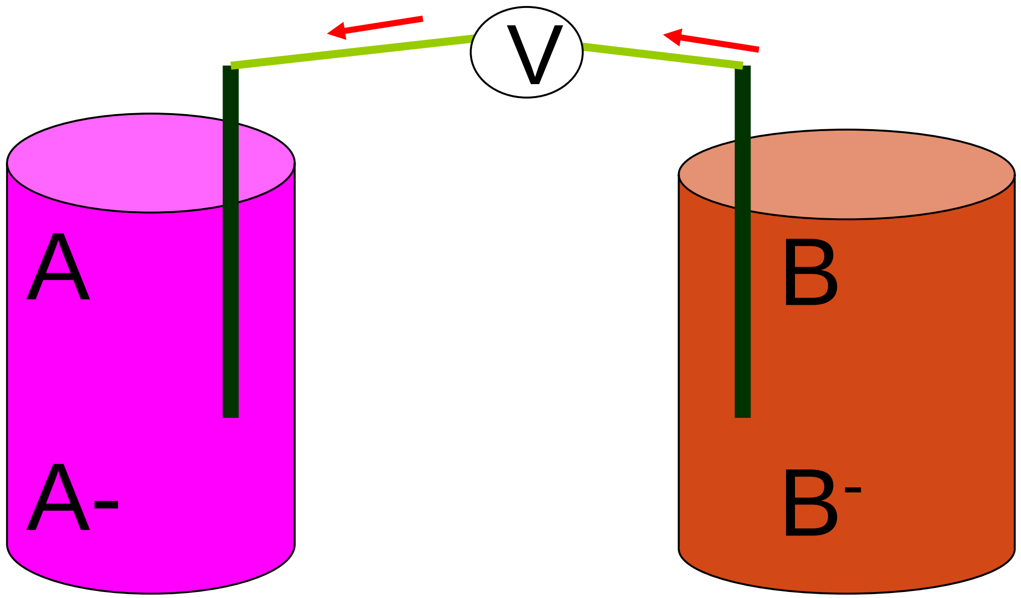
REDUCTION POTENTIAL AND DIRECTION OF THE REACTION

$$A + B^- \longrightarrow A^- + B \qquad \Delta G^\circ = -ve$$

B oxidized form

B^- reduced form

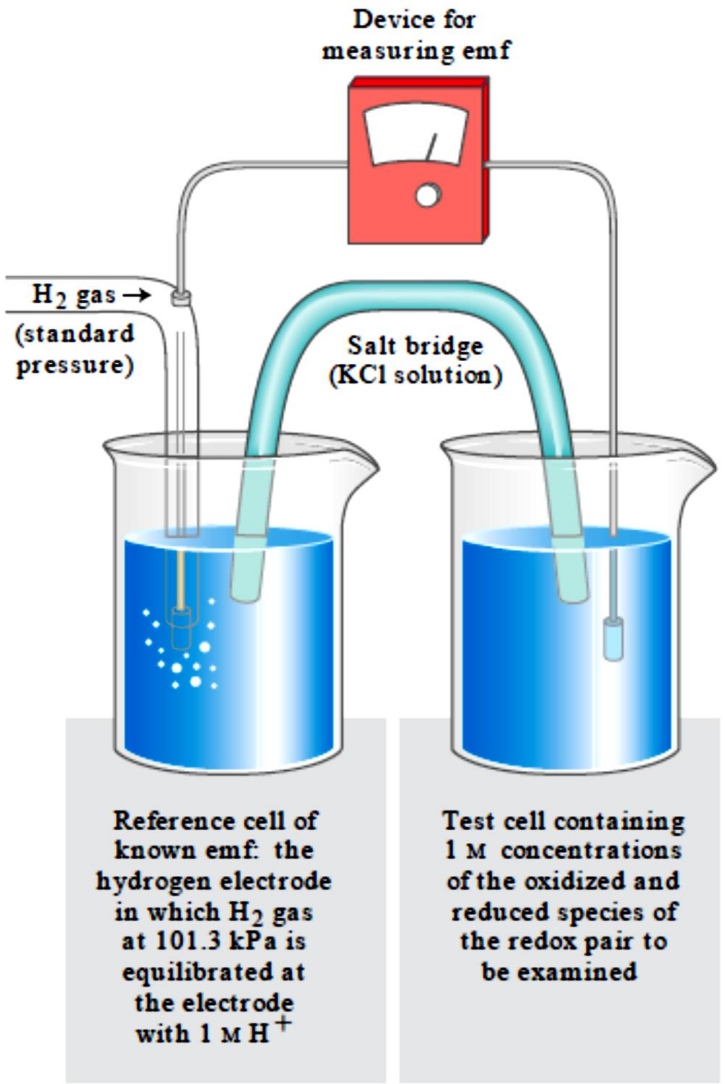
Redox couple



WHAT IS THE STANDARD?

- Hydrogen electrode
- H_2 Gas sensor

$$H^+ + e^- \longrightarrow \tfrac{1}{2}H_2$$



REDUCTION POTENTIAL AND DIRECTION OF THE REACTION

$$H^+ + X^- \longrightarrow H_2 + X \qquad \Delta G^o = -ve$$

X oxidized form

X^- reduced form

Redox couple

X^- has higher tendency to lose electrons than H_2 does

➔

Negative reduction potential

STANDARD REDUCTION POTENTIAL (E^0)

$$E = E^\circ + \frac{RT}{n\mathcal{F}} \ln \frac{[\text{electron acceptor}]}{[\text{electron donor}]}$$

$$E = E^\circ + \frac{0.026\ \text{V}}{n} \ln \frac{[\text{electron acceptor}]}{[\text{electron donor}]}$$

REDUCTION POTENTIAL

Oxidized + e^-	➔ Reduced	ΔE^0 (V)
Succinate	α ketoglutarate	- 0.67
Acetate	Acetaldehyde	- 0.60
NAD^+	**NADH**	**- 0.32**
Acetaldehyde	Ethanol	- 0.20
Pyruvate	Lactate	- 0.19
Fumarate	Succinate	+ 0.03
$Cytochrome^{+3}$	$Cytochrome^{+2}$	+ 0.22
oxygen	**water**	**+ 0.82**

TABLE 13–7 Standard Reduction Potentials of Some Biologically Important Half-Reactions, at pH 7.0 and 25 °C (298 K)

Half-reaction	$E^{\circ\prime}$ (V)
$\frac{1}{2}O_2 + 2H^+ + 2e^- \longrightarrow H_2O$	0.816
$Fe^{3+} + e^- \longrightarrow Fe^{2+}$	0.771
$NO_3^- + 2H^+ + 2e^- \longrightarrow NO_2^- + H_2O$	0.421
Cytochrome *f* (Fe^{3+}) + e^- $\longrightarrow$ cytochrome *f* (Fe^{2+})	0.365
$Fe(CN)_6^{3-}$ (ferricyanide) + e^- $\longrightarrow$ $Fe(CN)_6^{4-}$	0.36
Cytochrome a_3 (Fe^{3+}) + e^- $\longrightarrow$ cytochrome a_3 (Fe^{2+})	0.35
$O_2 + 2H^+ + 2e^- \longrightarrow H_2O_2$	0.295
Cytochrome *a* (Fe^{3+}) + e^- $\longrightarrow$ cytochrome *a* (Fe^{2+})	0.29
Cytochrome *c* (Fe^{3+}) + e^- $\longrightarrow$ cytochrome *c* (Fe^{2+})	0.254
Cytochrome c_1 (Fe^{3+}) + e^- $\longrightarrow$ cytochrome c_1 (Fe^{2+})	0.22
Cytochrome *b* (Fe^{3+}) + e^- $\longrightarrow$ cytochrome *b* (Fe^{2+})	0.077
Ubiquinone + $2H^+ + 2e^-$ $\longrightarrow$ ubiquinol + H_2	0.045
Fumarate^{2-} + $2H^+ + 2e^-$ $\longrightarrow$ succinate^{2-}	0.031
$2H^+ + 2e^- \longrightarrow H_2$ (at standard conditions, pH 0)	0.000
Crotonyl-CoA + $2H^+ + 2e^-$ $\longrightarrow$ butyryl-CoA	−0.015
Oxaloacetate^{2-} + $2H^+ + 2e^-$ $\longrightarrow$ malate^{2-}	−0.166
Pyruvate$^-$ + $2H^+ + 2e^-$ $\longrightarrow$ lactate$^-$	−0.185
Acetaldehyde + $2H^+ + 2e^-$ $\longrightarrow$ ethanol	−0.197
FAD + $2H^+ + 2e^-$ $\longrightarrow$ $FADH_2$	−0.219*
Glutathione + $2H^+ + 2e^-$ $\longrightarrow$ 2 reduced glutathione	−0.23
$S + 2H^+ + 2e^- \longrightarrow H_2S$	−0.243
Lipoic acid + $2H^+ + 2e^-$ $\longrightarrow$ dihydrolipoic acid	−0.29
$NAD^+ + H^+ + 2e^- \longrightarrow NADH$	−0.320
$NADP^+ + H^+ + 2e^- \longrightarrow NADPH$	−0.324
Acetoacetate + $2H^+ + 2e^-$ $\longrightarrow$ β-hydroxybutyrate	−0.346
α-Ketoglutarate + $CO_2 + 2H^+ + 2e^-$ $\longrightarrow$ isocitrate	−0.38
$2H^+ + 2e^- \longrightarrow H_2$ (at pH 7)	−0.414
Ferredoxin (Fe^{3+}) + e^- $\longrightarrow$ ferredoxin (Fe^{2+})	−0.432

CALCULATION OF ΔG^0 FROM ΔE^0

- $\Delta G^0 = - n \mathcal{F} \Delta E^0$ $\qquad \Delta G = -n\mathcal{F}\,\Delta E$ or $\Delta G'^\circ = -n\mathcal{F}\,\Delta E'^\circ$
  - $\mathcal{F}$ = Farady constant = 23.06 kcal/ *Volt*

- Calculate ΔG^0 of the following reaction

$$NADH + 1/2O_2 \longrightarrow NAD^+ + H_2O$$

$$NADH \longrightarrow NAD^+ + 2e^- \qquad \Delta E^0 = +0.32\ V$$

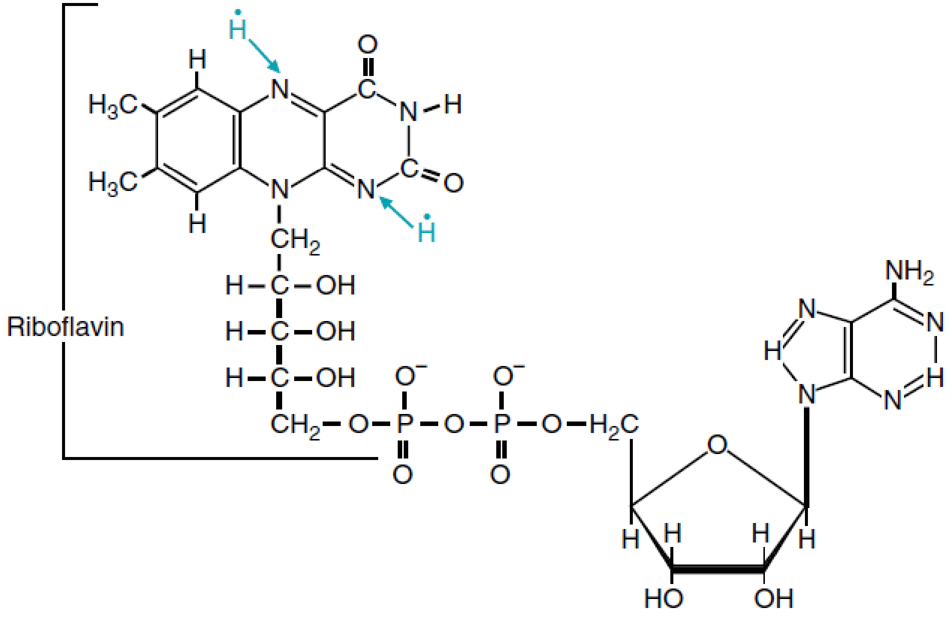
$$O + 2e^- \longrightarrow O^{2-} \qquad \Delta E^0 = +0.82\ V$$

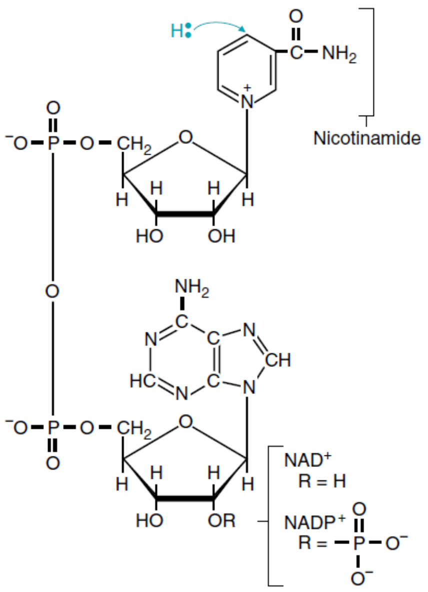
$$\Delta G^0 = -52.6\ kcal/mol$$

OXIDATION-REDUCTION REACTIONS (REDOX)

- $\Delta E = E_A - E_D$
- ΔE^o = at standard condition
- Does ΔE determine the feasibility of a reaction?

- $\Delta G^o = -nf\Delta E^o$
- In other words; energy (work) can be derived from the transfer of electrons Or
- Oxidation of food can be used to synthesize ATP





OXIDATION-REDUCTION REACTIONS (REDOX)

- Always involve a pair of chemicals: an electron donor and an electron acceptor (Food vs. NAD^+)
- NAD^+ vs. FAD
- NAD^+ vs. $NADP^+$ (fatty acid synthesis and detoxification reactions)