Nucleic Acids

F. Transcription
   1. Overview-mRNA structure
   2. Process
   3. RNA Polymerase
   4. Fidelity

G. Translation
   1. Genetic Code
      a. Triplet
      b. Deciphering
   2. tRNA
      a. Structure
      b. Anti-codon
      c. Acylation (charging)
         i. Aminoacyl-tRNA Synthetases
         ii. Mechanism
         iii. Fidelity
   3. Protein Biosynthesis
      a. Overview
      b. Process
      c. Ribosome review
      d. Peptidyl transferase
      e. fidelity

Lecture 31 (12/9/24)

- Reading: pp. 118-119,178,295,356,359, 530-531, 534-535,576,590,613-615,629,641-643
  Ch10; 356-360
  Ch17, 613-615
- Homework #34, 35 (last ones!)

NEXT
- Final Exam

Palmitate	16:0	Phosphatidic acid
Stearate	18:0	PE, PC, PS
Oleate	18:1 (Δ^9)	Sphingosine, ceramide
Linoleate	18:2 ($\Delta^{9,12}$)	Cholesterol
α-Linolenate	18:3 ($\Delta^{9,12,15}$)	
Arachidonate	20:4 ($\Delta^{5,8,11,14}$)	Recognize the 14 Vitamins

Lipids & Membranes

A. Lipids
   1. Roles
   2. Classes
      a. Fatty Acids
      b. Fats
      c. Waxes
      d. Membrane lipids
      e. Terpene

B. Membranes
   1. Introduction
   2. The 4 S's
      a. Size
      b. Solubility
      c. Shape
      d. Stability
   3. Models for Membrane structure
      a. Old Model
      b. Data
      c. Fluid Mosaic Model
      d. Testing the model
   4. The Red-Blood Cell Membrane
   5. Membrane Asymmetry
      a. Lipids
         i. Transverse
         ii. Lateral
      b. Protein
         a. Anchoring
         b. glycoproteins
   6. Membrane Fluidity

A. Vitamins & Cofactors
   1. Water soluble
   2. Fat Soluble

B. Metabolism
   1. Energetics
   2. Intermediates
      a. Redox; NADH & $FADH_2$
      b. Free energy; ATP cycle
      c. ATP
         i. Reaction
         ii. Energetics
         iii. Other high-energy
         iv. Energy charge

Vitamins & Cofactors

Vitamins & Cofactors

Vitamins

- Definition: essential nutrients required in the diet
- Different vitamins for different species
  - e.g., for rats & mice ascorbate is not a vitamin
  - *E. coli* do not have any vitamins
- Vitamins are classified as water soluble or fat soluble

Cofactors/Coenzymes

- Definition: low M_r molecules that act as carriers or give special functionality to enzymes
- Many coenzymes have vitamin molecules as their basis

Need to know: name, abbreviation, recognize structure, role in chemistry

Vitamins & Cofactors

Vitamins and Coenzymes

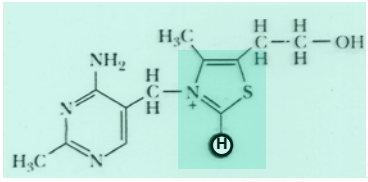
Vitamin	Coenzyme Form	Reaction mediated	Human Deficiency Disease
Water-Soluble			
① Thiamine (vitamin B_1)	Thiamine pyrophosphate	Acyl-group transfer	Beriberi
② Niacin (nicotinic acid)	Nicotinamide adenine dinucleotide (NAD^+) Nicotinamide adenine dinucleotide phosphate ($NADP^+$)	Re-dox	Pellagra
③ Riboflavin (vitamin B_2)	Flavin adenine dinucleotide (FAD) Flavin mononucleotide (FMN)	Re-dox	–
④ Pantothenic acid	Coenzyme A	Acyl-group transfer	–
⑤ Pyridoxal, pyridoxine, pyridoxamine (vitamin B_6)	Pyridoxal phosphate	Amino-group transfer	dermatitis
⑥ Cobalamin (vitamin B_{12})	5′-Deoxyadenosylcobalamin Methylcobalamin	Alkylation	Pernicious anemia
⑦ Biotin	Biotin-lysine complexes (biocytin)	Carboxylation	–
⑧ Lipoic acid	Lipoyl-lysine complexes (lipoamide)	Acyl-group transfer	–
⑨ Folic acid	Tetrahydrofolate	C1-group transfer	Megaloblastic anemia
⑩ Ascorbic acid (vitamin C)	Ascorbate	Re-dox	Scurvy
Fat-soluble			
① Retinol (vitamin A)	Retinal		Night blindness
② Ergocalciferol (vitamin D_2) Cholecalciferol (vitamin D_3)			Rickets
③ α-Tocopherol (vitamin E)			–
④ Vitamin K			Hemorrhage

Vitamins & Cofactors

① Vitamin B_1

Acyl-group transfer

Vitamin

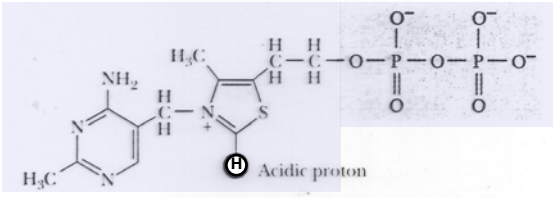


Thiamine (vitamin B_1)

Thiazole ring

In 1911, Casamir Funk realized that this "amine" was "vital" for life. He coined the term "vital-amine," which eventually was shortened to the word, "vitamin."

Coenzyme

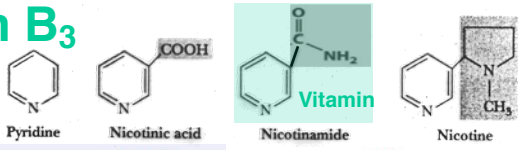


Thiamine pyrophosphate (TPP)

Vitamins & Cofactors

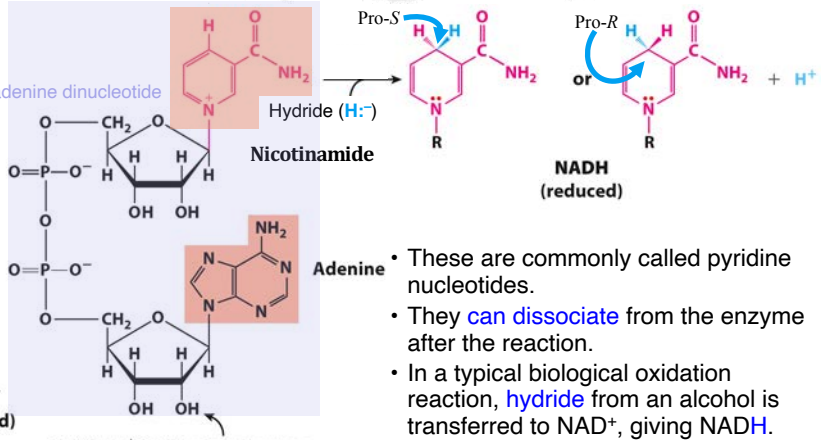
② Vitamin B_3 (aka Niacin)

Re-dox



Coenzyme

Nicotinamide adenine dinucleotide

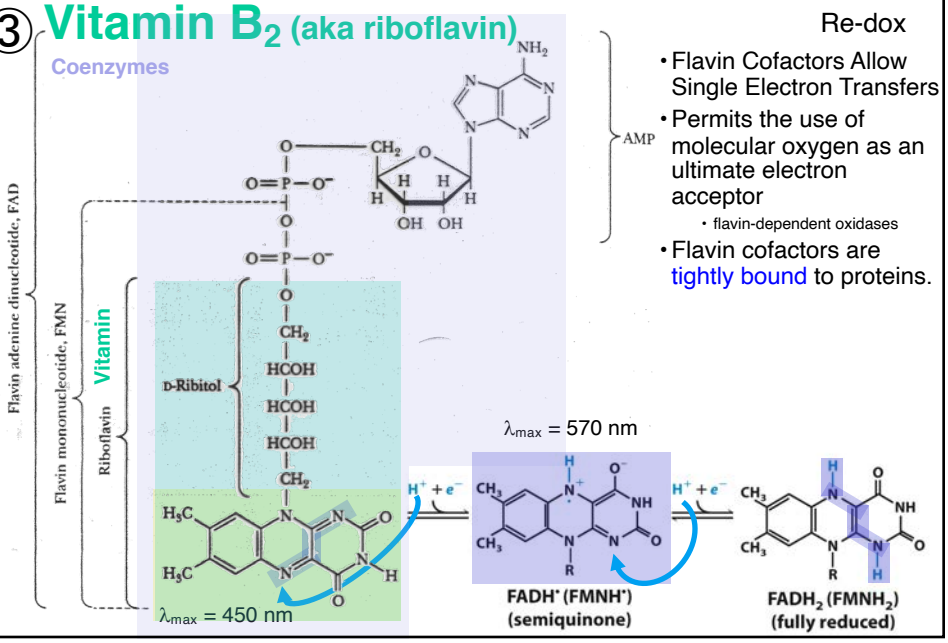


- These are commonly called pyridine nucleotides.
- They can dissociate from the enzyme after the reaction.
- In a typical biological oxidation reaction, hydride from an alcohol is transferred to NAD^+, giving NADH.
- Renamed **niacin** for **ni**cotinic **ac**id and vita**min**

Vitamins & Cofactors

③ Vitamin B_2 (aka riboflavin)

Coenzymes



Re-dox

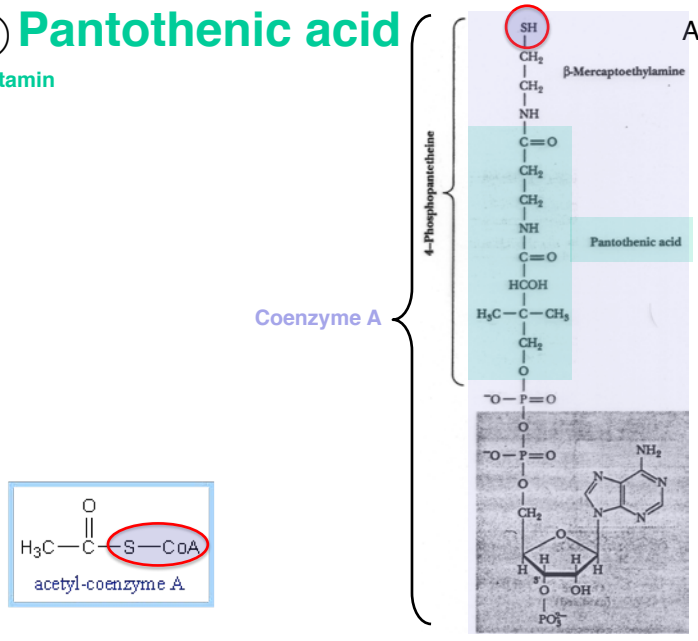
- Flavin Cofactors Allow Single Electron Transfers
- Permits the use of molecular oxygen as an ultimate electron acceptor
  - flavin-dependent oxidases
- Flavin cofactors are tightly bound to proteins.

Vitamins & Cofactors

④ Pantothenic acid

Vitamin

Acyl-group transfer

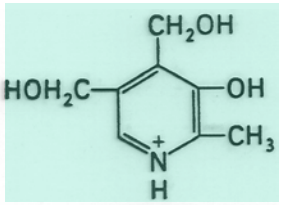


Vitamins & Cofactors

⑤ Vitamin B_6

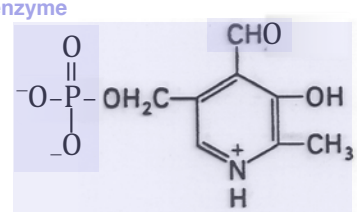
Amino-group transfer

Vitamin



Pyridoxine

Coenzyme



Pyridoxal 5-phosphate

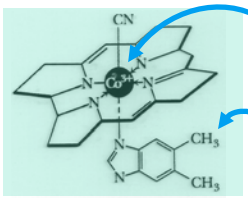
(PLP)

Vitamins & Cofactors

⑥ Vitamin B_{12}

Alkylation

Vitamin

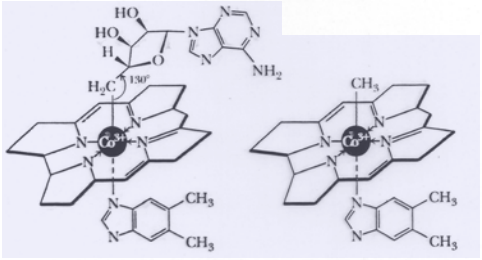


Cobalt ion

Dimethylbenzimidazole

Cyanocobalamin
Vitamin B_{12}

Coenzyme



5'-Deoxyadenosylcobalamin Methylcobalamin

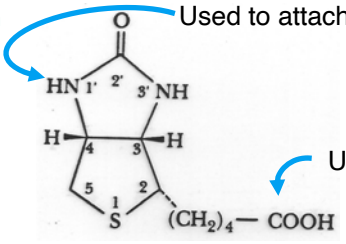
Coenzyme Forms

Vitamins & Cofactors

⑦ Biotin (aka Vitamin B_7)

Carboxylations

Vitamin

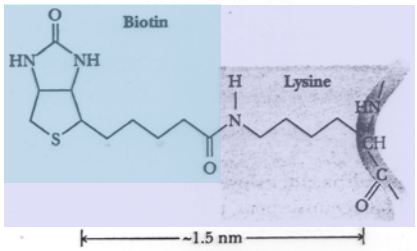


Used to attach carboxyl group ($-CO_2^-$)

Tightly binds Avidin and streptavidin (K_d=~10^{-15}); used in biotechnology

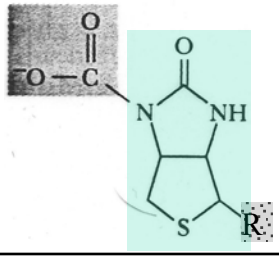
Used to attach to enzyme (Lys)

Coenzyme (covalent)



The biotin-lysine (biocytin) complex

Carboxylbiotin



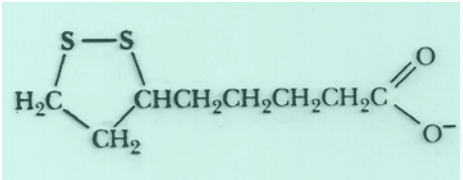
Vitamins & Cofactors

⑧ Lipoic Acid

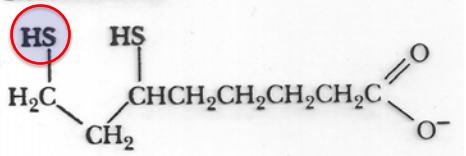
Acyl-group transfer

Vitamin

Lipoic acid, oxidized form

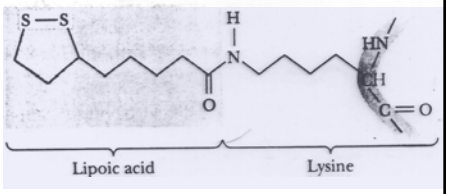


Reduced form



Coenzyme (covalent)

Lipoamide complex



Vitamins & Cofactors

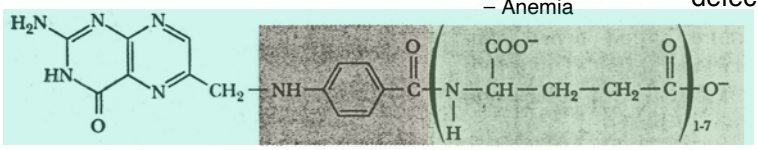
⑨Folic Acid (aka Vitamin B_9/B_{11})

One-carbon transfer

Vitamin

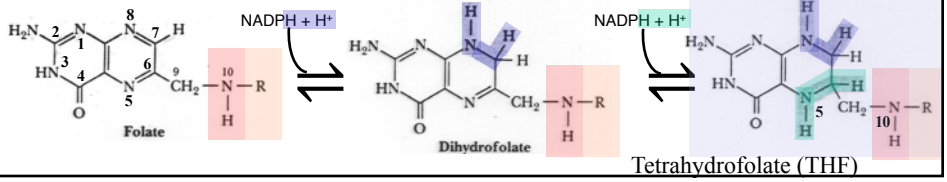
- Folate deficiency:
  - Diarrhea
  - Depression
  - Confusion
  - Anemia
- During pregnancy: fetal neural tube defects

Folic acid



Pterin (2-amino-4-oxopteridine) | *p*-Aminobenzoic acid (PABA) (was Vit. B_{10}) | Glutamates

Coenzyme



Folate | Dihydrofolate | Tetrahydrofolate (THF)

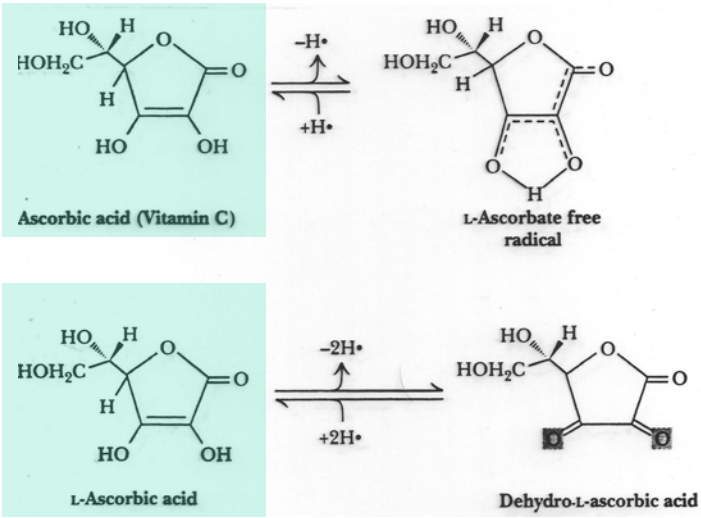
Vitamins & Cofactors

⑩ Ascorbic Acid (aka Vitamin C)

Re-dox

Vitamin

~~Coenzyme~~



Ascorbic acid (Vitamin C) | L-Ascorbate free radical | L-Ascorbic acid | Dehydro-L-ascorbic acid

Vitamins & Cofactors

Vitamins and Coenzymes

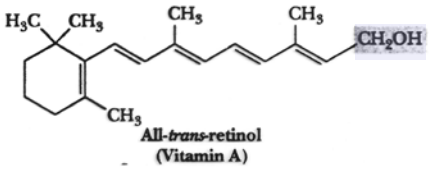
Vitamin	Coenzyme Form	Reaction mediated	Human Deficiency Disease
Water-Soluble			
① Thiamine (vitamin B_1)	Thiamine pyrophosphate	Acyl-group transfer	Beriberi
② Niacin (nicotinic acid)	Nicotinamide adenine dinucleotide (NAD^+) Nicotinamide adenine dinucleotide phosphate ($NADP^+$)	Re-dox	Pellagra
③ Riboflavin (vitamin B_2)	Flavin adenine dinucleotide (FAD) Flavin mononucleotide (FMN)	Re-dox	–
④ Pantothenic acid	Coenzyme A	Acyl-group transfer	–
⑤ Pyridoxal, pyridoxine, pyridoxamine (vitamin B_6)	Pyridoxal phosphate	Amino-group transfer	dermatitis
⑥ Cobalamin (vitamin B_{12})	5′-Deoxyadenosylcobalamin Methylcobalamin	Alkylation	Pernicious anemia
⑦ Biotin	Biotin-lysine complexes (biocytin)	Carboxylation	–
⑧ Lipoic acid	Lipoyl-lysine complexes (lipoamide)	Acyl-group transfer	–
⑨ Folic acid	Tetrahydrofolate	C1-group transfer	Megaloblastic anemia
⑩ Ascorbic acid (vitamin C)	Ascorbate	Re-dox	Scurvy
Fat-soluble			
① Retinol (vitamin A)	Retinal		Night blindness
② Ergocalciferol (vitamin D_2) Cholecalciferol (vitamin D_3)			Rickets
③ α-Tocopherol (vitamin E)			–
④ Vitamin K			Hemorrhage

Vitamins & Cofactors

Fat Soluble

① Vitamin A

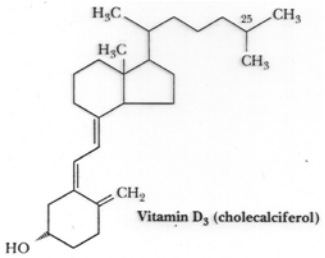
Light absorption



All-*trans*-retinol
(Vitamin A)

② Vitamin D_3

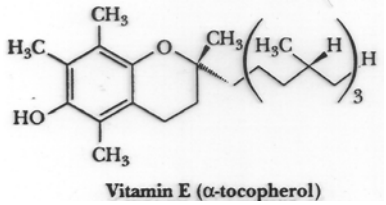
Calcium homeostasis



Vitamin D_3 (cholecalciferol)

③ Vitamin E

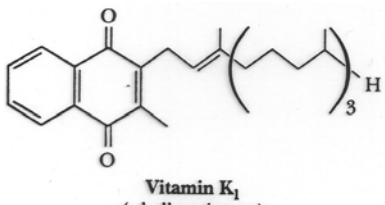
Redox



Vitamin E (α-tocopherol)

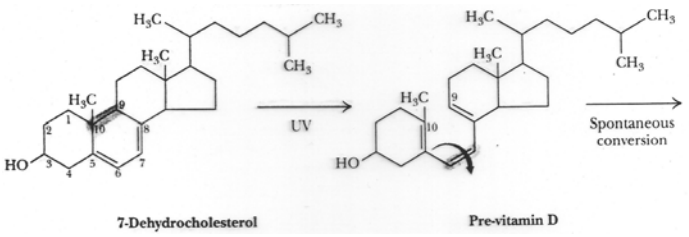
④ Vitamin K

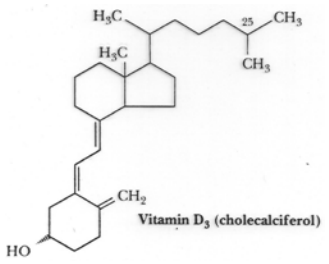
Blood clotting



Vitamin K_1
(phylloquinone)

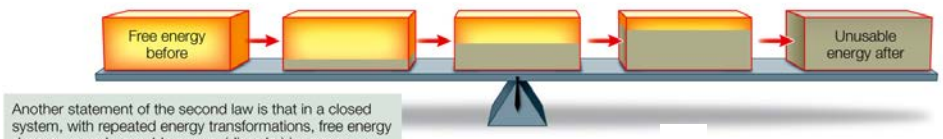
Vitamins & Cofactors





Overview of Metabolism

Recall from First lecture: Energetics of Life



Another statement of the second law is that in a closed system, with repeated energy transformations, free energy decreases and unusable energy (disorder) increases—a phenomenon known as the increase in **entropy**.

Chaos $\rightleftharpoons$ Cell

ΔS_{system} is $\ominus$

$$\Delta S_{universe} = \Delta S_{system} + \Delta S_{surroundings}$$

$\oplus$ $\quad$ $\ominus$ $\quad$ $\oplus$

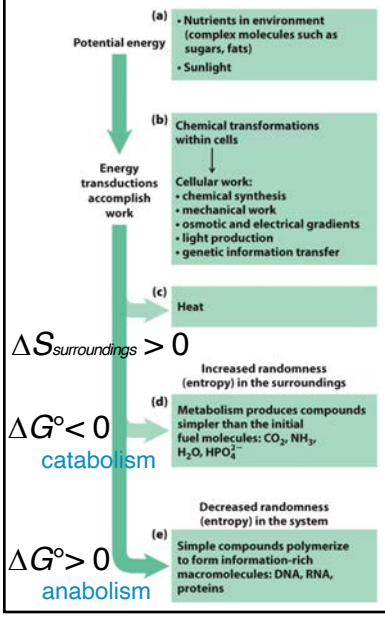
- As the entropy of the universe increases, creating and maintaining order requires work and energy.
- Living organisms exist in a dynamic steady state and are never at equilibrium with their surroundings.
- Energy **coupling** allows living organisms to transform energy, releasing much of it to the surroundings.

Recall from First lecture: 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.

Organisms Use the First Law Big-Time (perform energy transformations) to Stay Alive



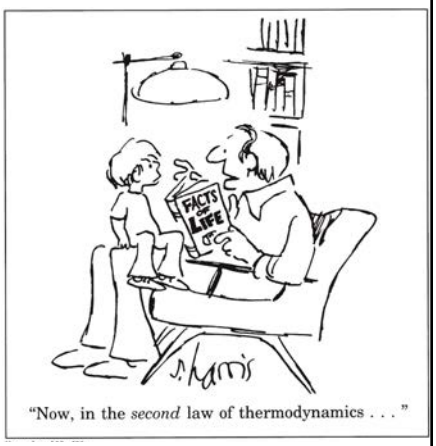
Favorable and Unfavorable Reactions

- The breakdown of some metabolites releases a significant amount of free 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 free energy (**endergonic**).
  - A reaction might be thermodynamically unfavorable ($\Delta G° > 0$).
    - Creating order requires work and energy.

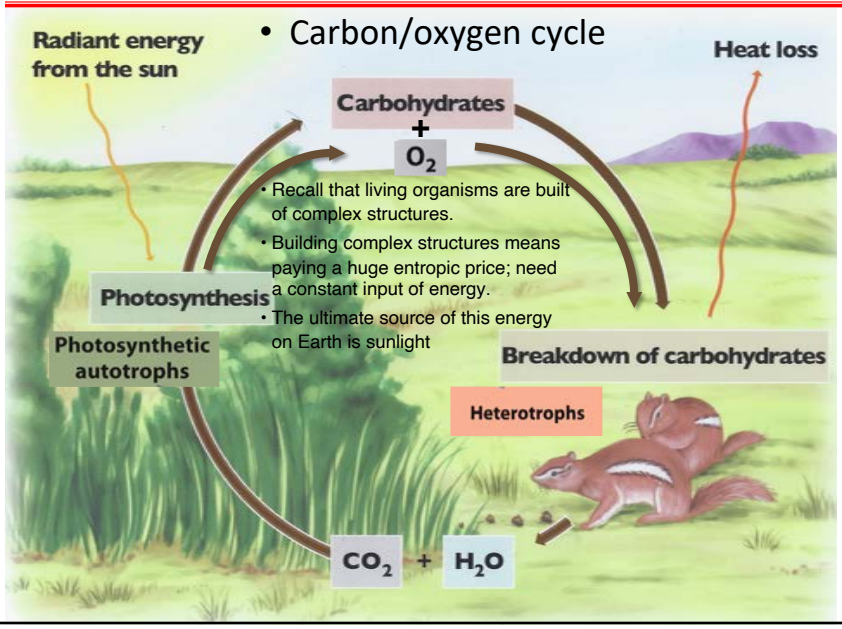
Metabolism

Issues:

- Thermodynamics and biochemistry; carbon/oxygen cycle & nitrogen cycle
- Common organic-chemical 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.

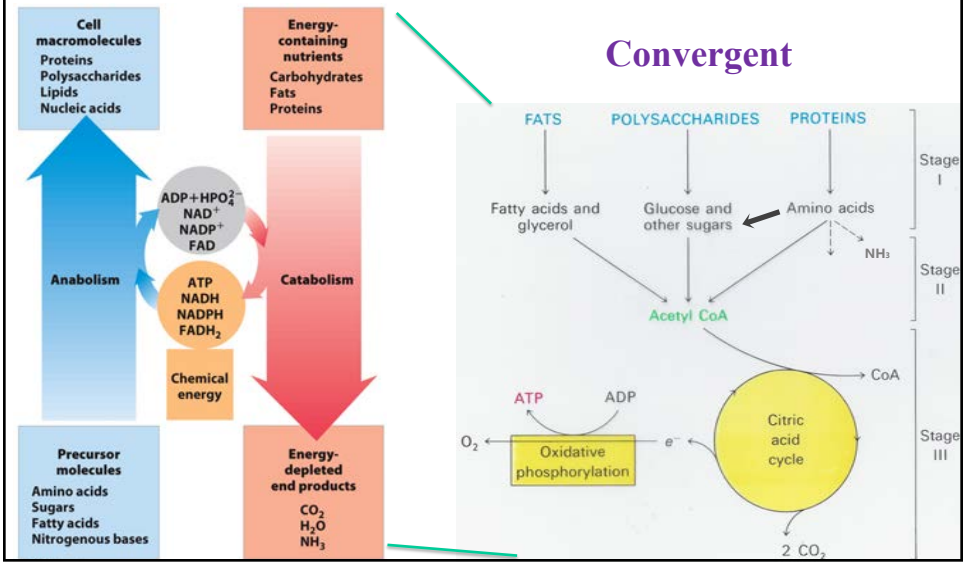


Metabolism

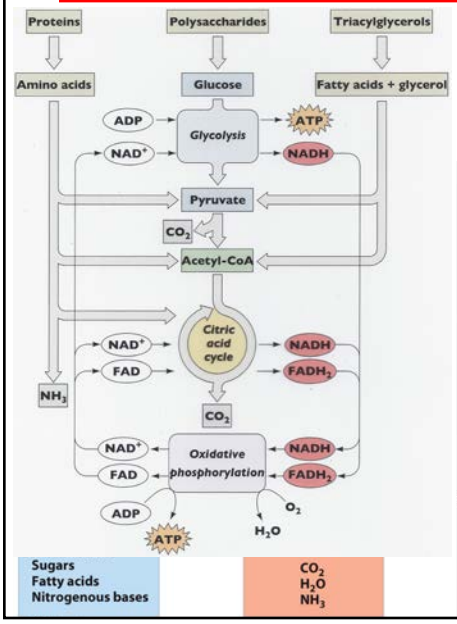


Metabolism

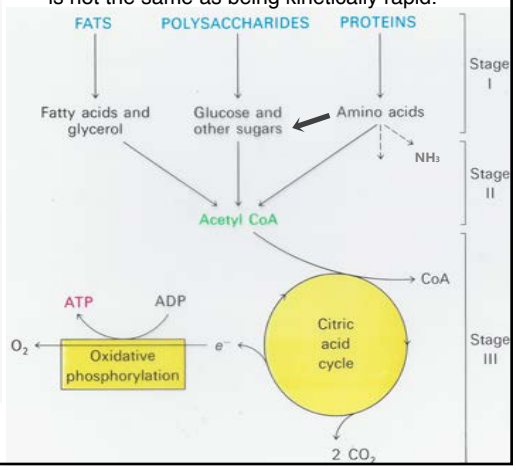
Metabolism is the Sum of All Chemical Reactions in the Cell



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.



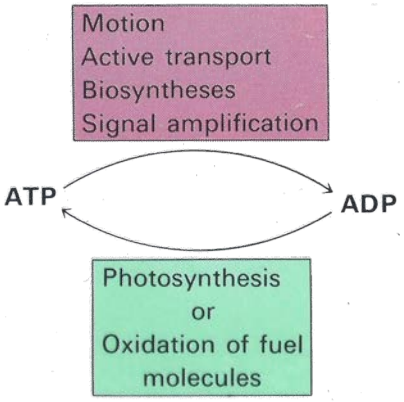
Metabolism

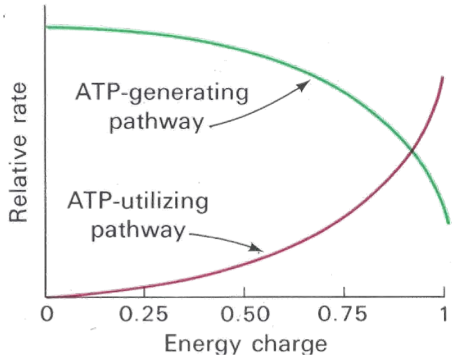
NAD and NADP Are Common Redox Cofactors

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

Metabolism

THE ATP CYCLE



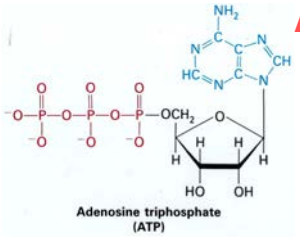


This "buffering" of energy in the cell keeps the [ATP] high enough to keep fighting the second law of thermodynamics.

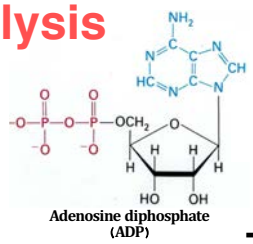
$$\text{Energy Charge} = \frac{[ATP^{-4}] + \tfrac{1}{2}[ADP^{-3}]}{[ATP^{-4}] + [ADP^{-3}] + [AMP^{-2}]}$$

Metabolism

ATP Hydrolysis



+ H_2O ⇌



+ 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

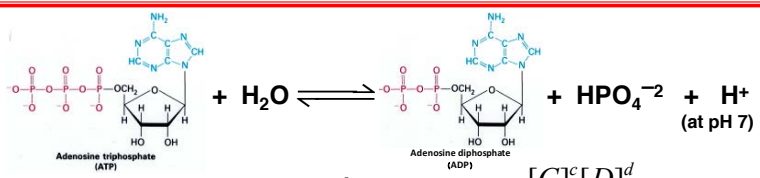
Also, recall that the actual free-energy change of a reaction depends on:

- 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

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

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

$$\Delta G' = \Delta G^{\circ\prime} + RT \ln K'_{eq}$$

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

For the above reaction (hydrolysis of ATP):

$$\Delta G^{\circ\prime} = -7.3 \text{ kcal/mole}$$

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

When K'_{eq} is . . .	$\Delta G^{\circ\prime}$ 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

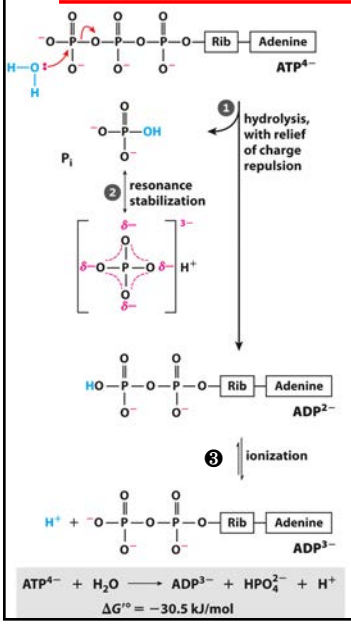
What makes the $\Delta G^{0\prime}$ of ATP so high?

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

K'_{eq}	$\Delta G^{\circ\prime}$ (kJ/mol)	$\Delta G^{\circ\prime}$ (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

* Although joules and kilojoules are the standard units of energy and are used throughout this text, biochemists and nutritionists sometimes express $\Delta G^{\circ\prime}$ 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

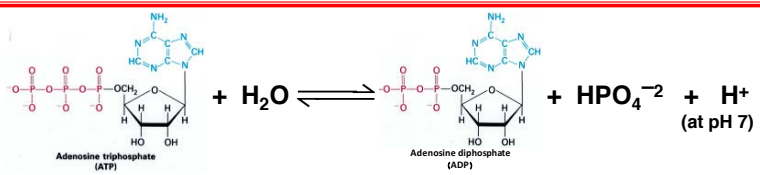


- Contributions of the incredible favorability of the ATP hydrolysis reaction:
  - Better charge separation in products (relief of charge repulsion)
  - More favorable resonance stabilization of products
  - Ionization of products (ADP or P_i)

$H_2PO_4^- \rightleftharpoons HPO_4^{2-} + H^+$ (at pH 7)

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

Metabolism



$+ H_2O \rightleftharpoons + HPO_4^{-2} + H^+$ (at pH 7)

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

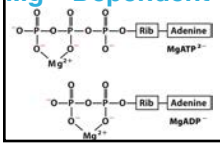
In RBCs = 1.5×10^{-4} M

$\Delta G = \Delta G^{\circ\prime} + RT \ln \frac{[MgADP^-] \cdot [P_i]}{[MgATP^{2-}]}$

$\Delta G' = \Delta G^{\circ\prime} + RT \ln \frac{[ADP^{-3}]\,[HPO_4^{-2}]}{[ATP^{-4}]}$

At 25 °C: $\Delta G' = \Delta G^{\circ\prime} + 0.59 \ln \frac{[ADP^{-3}]\,[HPO_4^{-2}]}{[ATP^{-4}]}$

$\Delta G^{\circ\prime}$ of ATP Hydrolysis is Mg^{++} Dependent



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

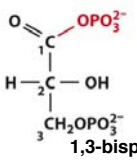
WOW!

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

Are there other compounds in the cell with high negative $\Delta G^{\circ\prime}$ of hydrolysis?

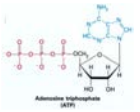
$\Delta G' = -12.5$ kcal/mole

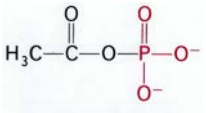
Metabolism



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

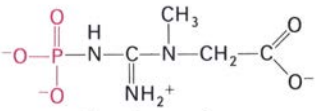
1,3-bisphosphoglycerate





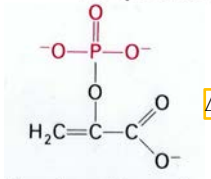
$\Delta G^{\circ\prime} = -11.8$ kcal/mole

Acetyl phosphate



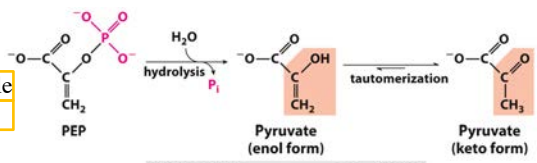
$\Delta G^{\circ\prime} = -10.3$ kcal/mole

Phosphocreatine



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

Phosphoenolpyruvate (PEP)

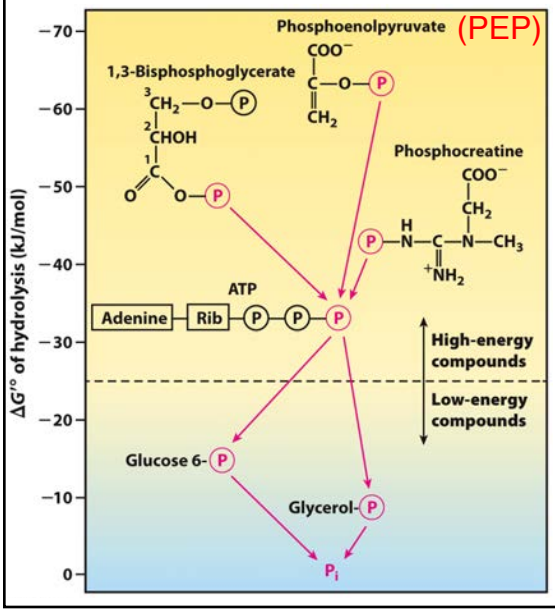


$PEP^{3-} + H_2O \longrightarrow pyruvate^- + HPO_4^{2-}$
$\Delta G'^{\circ} = -61.9$ kJ/mol

TABLE 13-4 Standard Free-Energy Changes of Some Chemical Reactions

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

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 $\Delta G^{\circ\prime}$.

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.

End of BB 421

- TODAY – Course Evaluation
  bu.edu/courseeval, email, or Bb

- Review session – Thursday 5:00-6:00 PM in HAR-208.
  For cumulative questions

- Review session – next Monday 4:00-5:45 PM in HAR-208.

- ***Final Exam*** *– December 18, Wednesday 8-11 AM in MORSE.*