

### Bioenergetics

- Energy is the capacity or ability to do work
- All organisms need a constant supply of energy for functions such as motion, transport across membrane barriers, synthesis of biomolecules, information transfer, maintain body temperature etc. The cell can transform chemical energy to mechanical or potential energy.
- Some organisms such as plants are able to utilize light energy from the sunlight and  $\text{CO}_2$  as a source of carbon by the process known as photosynthesis. These organisms are called as Phototrophs.  $\text{CO}_2 + \text{H}_2\text{O} = \text{organic compounds} + \text{O}_2$
- Chemotrophs are organisms that utilize the organic material synthesized by autotrophs as a source of energy.  $\text{Organic compounds} + \text{O}_2 = \text{CO}_2 + \text{H}_2\text{O} + \text{energy}$ .
- This leads to the cycling of carbon and oxygen between phototrophs, chemotrophs and the environment.

### Free Energy Change

- The free energy change ( $\Delta G$ ) of a chemical process is a measure of the energy available to do work.
- Standard free energy change  $\Delta G^\circ$  is when reaction conditions are standard: T is 25 °C, P = 1 atm, conc of all reactants is 1M.
- For biochemical reactions, the pH has to be 7. The standard biochemical free energy change is designated as  $\Delta G^{\circ'}$
- $\Delta G = G_{\text{products}} - G_{\text{reactants}}$ .
- If  $\Delta G < 0$ , the forward reaction is favored relative to the reverse reaction. The products are more stable than the reactants. As reaction proceeds, energy is released, it can be used to do work
- If  $\Delta G = 0$ , reactants and products are at equilibrium
- if  $\Delta G > 0$ , reactants are at lower energy than products; energy needs to be supplied for the reaction to proceed.

### Free Energy and Reaction Equilibrium

$\Delta G^{o'}$  is directly dependent on the equilibrium constant  $K'_{eq}$ .

For a reaction:  $A \leftrightarrow B$ ,  $\Delta G = \Delta G^{o'} + RT \ln [B]/[A]$

$\Delta G$  depends on  $\Delta G^{o'}$  as well as the concentration of the reactant and products

at equilibrium,  $\Delta G = 0$ , and  $K'_{eq} = [B]/[A]$

$\Delta G^{o'} = -RT \ln K'_{eq}$  or  $\Delta G^{o'} = -2.303 RT \log K'_{eq}$

since  $R = 1.987 \times 10^{-3} \text{ kcal.mol}^{-1}.\text{deg}^{-1}$  and  $T = 298\text{K}$ ,

$\Delta G^{o'} = -1.36 \log K'_{eq}$  or  $K'_{eq} = 10^{-\Delta G^{o'} / 1.36}$

Based on this relationship, lower the value of  $\Delta G^{o'}$ , higher the  $K'_{eq}$  and formation of B is favored

### Coupled Reactions

- Consider the earlier example:  $A \leftrightarrow B$
- $K'_{eq} = 10^{-\Delta G^{o'} / 1.36}$
- If  $\Delta G^{o'} = 4 \text{ kcal.mol}^{-1}$ , then  $K'_{eq} = 1.15 \times 10^{-3}$  and equilibrium is reached at very low [B].
- If this reaction is coupled to another reaction that has a very low (negative)  $\Delta G^{o'}$ , then overall  $\Delta G^{o'}$  of coupled reaction is lowered such that  $K'_{eq}$  is increased significantly
- Biochemical reactions are usually coupled to the hydrolysis of ATP:  
 $\text{ATP} \leftrightarrow \text{ADP} + \text{P}_i$   $\Delta G^{o'} = -7.3 \text{ kcal.mol}^{-1}$
- Overall  $\Delta G^{o'} = 4 + (-7.3) = -3.3 \text{ kcal.mol}^{-1}$  and  $K'_{eq} = 267$
- Overall  $K'_{eq} = [B]/[A] \times [\text{ADP}][\text{P}_i]/[\text{ATP}] = 267$
- In the cell,  $[\text{ATP}]/[\text{ADP}][\text{P}_i]$  is maintained at  $500 \text{ M}^{-1}$ , therefore  $K'_{eq} = [B]/[A] \times (1/500) = 267$  or  $[B]/[A] = 267 \times 500 = 1.34 \times 10^5$
- A and B can represent biomolecules, different conformations of the same protein, ion concentration across a membrane etc.

### Coupled Reactions (continued)

- Two or more reactions can be coupled when one or more products of one reaction are reactants of the next reaction.
- When multiple reactions are coupled, it becomes a pathway.
- Coupling reactions allow for unfavorable reactions (with a positive  $\Delta G^{\circ}$ ) to proceed.  
$$\text{Glc} + \text{Pi} = \text{Glc-6-PO}_4 + \text{H}_2\text{O} \quad \Delta G^{\circ} = +14 \text{ kJ/mol}$$
- $$\text{ATP} + \text{H}_2\text{O} = \text{ADP} + \text{Pi} \quad \Delta G^{\circ} = -31 \text{ kJ/mol}$$
- Total: 
$$\text{Glc} + \text{ATP} = \text{Glc-6-PO}_4 + \text{ADP} \quad \Delta G^{\circ} = -17 \text{ kJ/mol}$$
- The direct phosphorylation of glucose would not proceed spontaneously because of the positive  $\Delta G^{\circ}$ .

### ATP serves as a high-energy compound

- ATP has 2 phosphoanhydride bonds and one phosphoester bond.
- The phosphoanhydride bond has a high free energy change of hydrolysis. The  $\Delta G^{\circ}$  for hydrolysis of ATP is  $-30 \text{ kJ/mol}$ . ( $-7.3 \text{ kcal/mol}$ )
- $\Delta G^{\circ}$  for ATP hydrolysis is more negative than most biochemical reactions and than hydrolysis of phosphorylated compounds such as Glc-6-PO<sub>4</sub> or Glycerol-3-PO<sub>4</sub>. This means that ATP has a higher phosphoryl transfer potential than these compounds.
- The products of ATP hydrolysis, ADP and Pi are much more stable than ATP. Factors that make ADP + Pi more stable than ATP: Resonance structures of Pi, Repulsion between the 4 negative charges of ATP and water readily hydrates ADP + Pi.

### Comparison of Phosphoryl Transfer Potential of Compounds

- Phosphoenolpyruvate  $\leftrightarrow$  Pyruvate + Pi  $\Delta G^{\circ} = -62$  kJ/mol
- Creatine phosphate  $\leftrightarrow$  creatine + Pi  $\Delta G^{\circ} = -43$  kJ/mol
- ATP  $\leftrightarrow$  ADP + Pi  $\Delta G^{\circ} = -31$  kJ/mol
- Glucose-6-phosphate  $\leftrightarrow$  glucose + Pi  $\Delta G^{\circ} = -14$  kJ/mol
  
- ATP is ranked in the middle on such a comparison. This allows ATP to phosphorylate compounds ranked lower than itself. It is favorable to synthesize ATP from ADP by higher ranked PEP and CP.
- It is harder to synthesize the highest ranking molecules and the lowest ranking ones don't release enough energy to perform significant work. Thus, ATP's intermediate ranking is ideal for its function as the energy currency of the cell

### High Energy Bonds: Phosphate Anhydrides

- **Phosphate anhydride bonds.** Examples are ATP and other nucleoside triphosphates. The phosphate anhydride bond is the bond between two phosphates. all NTPs have 2 high energy bonds. (N = any nucleoside)
- NTP + H<sub>2</sub>O = NDP + Pi  $\Delta G^{\circ} = -31$  kJ/mol
- NDP + H<sub>2</sub>O = NMP + Pi  $\Delta G^{\circ} = -31$  kJ/mol
- NTP + H<sub>2</sub>O = NMP + P<sub>2</sub> i  $\Delta G^{\circ} = -31$  kJ/mol
- NMP + H<sub>2</sub>O = N + Pi  $\Delta G^{\circ} = -14$  kJ/mol

### High Energy Bonds: Mixed anhydrides

- Mixed anhydrides or carboxylate-phosphate anhydrides. This is when a phosphate group is attached to a carboxylic acid group. General formula:  $R-COO-PO_3-R'$

- an example is an intermediate of the glycolytic pathway: 1,3 biphosphoglycerate.



- In the glycolysis pathway, the hydrolysis of 1,3-BPG is coupled to the formation of a molecule of ATP.



So adding up:  $1,3 \text{ BPG} + \text{ADP} = 3\text{-phosphoglycerate} + \text{ATP}$   
 $\Delta G^{\circ} = -19 \text{ kJ/mol}$

### High Energy Bonds: Enoyl Phosphates

- Enoyl phosphates. The keto group can exist in its tautomeric form called as the enol form.
- $R-CH=O \leftrightarrow R=C-OH$ . The enol form can form an ester linkage with a phosphate; this kind of a bond is called as an enoyl phosphate bond. Hydrolysis of this bond has a highly negative change in free energy.

specific example: an intermediate of glycolysis.

Phosphoenolpyruvate (PEP)

- $PEP + \text{H}_2\text{O} = \text{Pyruvate (enol form)} + \text{Pi} \quad \Delta G^{\circ} = -60 \text{ kJ/mol}$

### High Energy Bonds: Thioesters

- Thioester bonds. The general formula is R-C(=O)-S-R'.
- Hydrolysis leads to formation of a carboxylic acid and a sulphhydryl group.
- $\text{R-C(=O)-S-R}' + \text{H}_2\text{O} = \text{RCOOH} + \text{R}'\text{-SH} \quad \Delta G^{\circ} = -30 \text{ kJ/mol}$
- A common thioester encountered in metabolic pathways, particularly in fatty acid oxidation is acetyl-S-CoA.
- $\text{CH}_3\text{-C(=O)-S-CoA} + \text{H}_2\text{O} = \text{CH}_3\text{COOH} + \text{CoA-SH}$   
 $\Delta G^{\circ} = -30 \text{ kJ/mol}$

### Oxidation

- Catabolism is the oxidation of Carbon to CO<sub>2</sub>.
- The energy stored in carbon is dependent on its oxidation number. Lower the oxidation number, more negative the free energy of oxidation.
- Catabolism proceeds in multiple steps; the released energy is sequestered in metabolic intermediates and in reduced compounds NADH and FADH<sub>2</sub>.
- Compounds with high phosphoryl transfer potential synthesize some ATP
- Most of the ATP synthesis is coupled with oxidation of these reduced compounds by a process called as oxidative phosphorylation. As electrons are transferred down electron carriers, a proton gradient is generated which drives ATP synthesis.

### Stages of Catabolism

- 3 stages of extraction of energy from carbohydrates and lipids. Proteins usually not used for energy, rather for anabolic processes
- Stage I is the hydrolysis of all classes of molecules to their respective monomers. No energy is made available in this step
- Stage II involves the conversion of fats and sugars to acetyl CoA, a 2-carbon metabolite. Some ATP is generated in stage II
- Stage III is the complete oxidation of acetyl CoA to CO<sub>2</sub> by the TCA cycle. The reducing power of carbon is captured in NADH and FADH<sub>2</sub>. The electrons are transferred via a series of electron carriers to the final acceptor O<sub>2</sub> and ATP is synthesized by oxidative phosphorylation.

### Activated Electron Carriers

- NADH and FADH<sub>2</sub> serve as electron carriers for most metabolic pathways.
- They are vitamin cofactors and also nucleotides. NAD<sup>+</sup> and FAD are derivatives of niacin and riboflavin respectively
- NAD<sup>+</sup> accepts one electron and one H to become NADH in oxidation (dehydrogenation) of -CHOH to -C=O
- FAD is reduced to FADH<sub>2</sub> by accepting 2 H in dehydrogenation of -CH-CH- to -C=C-
- Certain anabolic pathways, such as the pentose phosphate pathway and FA biosynthesis, use NADP<sup>+</sup>/ NADPH instead of NAD<sup>+</sup>/ NADH

### Activated Carrier of Carbon Metabolites

- Coenzyme A is an activated carrier of acyl groups during FA oxidation. CoA also serves as a carrier for the 2 carbon (acetyl) fragments that enter the TCA cycle.
- CoA is a derivative of the vitamin pantothenic acid
- The acyl group is covalently attached by a high energy thioester linkage. Hydrolysis of the thioester has a  $\Delta G^{\circ} = -31 \text{ kJ/mol}$
- During FA biosynthesis, another activated molecule called as acyl carrier protein (ACP) is used as a carbon carrier.
- The activated carriers stabilizes the metabolites thermodynamically and traps them as intermediates of specific pathways

### Recurring reactions in metabolic pathways

- **Oxidation-Reduction** : Catalyzed by dehydrogenases
- **Group transfer reactions**: most common type is transfer of phosphate or acyl groups. Amine groups in aa metabolism
- **Hydrolysis reactions**: during stage I of catabolism.
- **Cleavage reactions**: formation of simpler compounds by breaking C-C bond. Dehydration to form C=C also common
- **Isomerization reactions**: Rearrangements are usually to facilitate the next step.
- **Energy requiring bond formation or breakage**: Usually involves the addition or removal of C as  $\text{CO}_2$ .



### Regulation of Metabolism

- Enzyme levels (mass) are regulated by altering rate of transcription or rate of degradation
- Allosteric control regulates enzyme activity. This is called feed-back inhibition or feed-forward activation. Activity is also regulated by reversible covalent modification triggered by hormones and signal transduction.
- Segregation of substrate and enzyme in different compartments adds a level of regulation. Biosynthetic and degradative pathways are always in different compartments. Specific transporters are responsible for making substrates accessible.
- Energy charge of cell: can be from 0 to 1.  $([ATP] + \frac{1}{2}[ADP])/([ATP]+[ADP]+[AMP])$  Higher EC favors biosynthesis and lower EC favors catabolism.
- Metabolism is sensitive to the ratio of NADH and NAD<sup>+</sup>. A deficit of NADH promotes catabolic activity