

## Biochemistry 462a – Bioenergetics

Reading - Lehninger Principles, Chapter 14, pp. 485-512

Practice problems - Chapter 14: 2 - 8, 10, 12, 13; Physical Chemistry extra problems, free energy problems

### Free Energy

- Bioenergetics is the quantitative study of how biological systems gain and use energy - it is part of thermodynamics. Bioenergetics is essential for
  - understanding how metabolic processes provide energy for the cell
  - understanding the structure of macromolecules
  - understanding how membrane transport occurs
- In short, for understanding all the fundamental processes that define biochemistry. We shall specifically be concerned in using bioenergetics to describe conditions under which processes occur spontaneously. (See html notes on web for what "spontaneous" reaction means.)
- **Although we can use bioenergetics to determine whether a process will occur spontaneously, bioenergetics gives us no information as to how fast the process will occur.**
- The thermodynamic function that is most useful for biochemistry is the **Gibbs Free Energy**,  $G=H-TS$ , which combines
  - **Enthalpy, H**, a measure of the energy of the system at constant pressure, and
  - **Entropy, S**, a measure of the randomness of the system.
- For any process,
  - If  $\Delta H$  is **negative**, then heat is released - this a favorable enthalpy change.
  - If  $\Delta S$  is **positive**, then the randomness of the system increases - this is a favorable entropy change.
- For any process, the change in free energy is given by  $\Delta G=\Delta H-T\Delta S$ .
  - **If  $\Delta G$  is negative**, the process is spontaneous.
  - **If  $\Delta G=0$** , the process is at equilibrium.
  - **If  $\Delta G$  is positive**, the process is not spontaneous (in fact the reverse process is spontaneous).
  - **The value and sign of  $\Delta G$  depends on the interplay of enthalpy and entropy.** Just because  $\Delta H$  is negative doesn't mean that  $\Delta G$  will be negative and just because  $\Delta S$  is positive doesn't mean that  $\Delta G$  will be negative.
- Consider the melting of ice and the values of  $\Delta G$ ,  $\Delta H$ ,  $\Delta S$  at various temperatures.
  - For ice melting  $\Delta H$  is positive (unfavorable) because one is breaking hydrogen bonds.
  - $\Delta S$  is positive (favorable) because the water molecules are more disordered in water than in ice.

Temperature	$\Delta H$	$-T\Delta S$	$\Delta G$	What Happens?
+10°C	+6.4 kJ/mol	-6.6 kJ/mol	-0.2 kJ/mol	Ice melts
0°C	+6.0 kJ/mol	-6.0 kJ/mol	0 kJ/mol	Ice and water coexist
-10°C	+5.6 kJ/mol	-5.4 kJ/mol	+0.2 kJ/mol	Water freezes

- In the table below are examples which illustrate how  $\Delta H$  and  $\Delta S$  interact to produce a favorable  $\Delta G$ .

Reaction	$\Delta H$	$-T\Delta S$	$\Delta G$	Comment
$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$	-82 kJ/mol	-136 kJ/mol	-218 kJ/mol	$\Delta H$ favors; $\Delta S$ favors
$C_2H_5OH + 3O_2 \rightarrow 2CO_2 + 3H_2O$	-1367 kJ/mol	+41 kJ/mol	-1326 kJ/mol	$\Delta H$ favors; $\Delta S$ opposes
$N_2O_5 \rightarrow 2NO_2 + 1/2O_2$	+110 kJ/mol	-140 kJ/mol	-30 kJ/mol	$\Delta H$ opposes; $\Delta S$ favors

- Temperature also plays a critical role in determining the sign of  $\Delta G$  ( $\Delta G = \Delta H - T\Delta S$ ).

$\Delta H$	$\Delta S$	Low Temperature	High Temperature
+	+	$\Delta G$ +; not favored	$\Delta G$ -; favored
+	-	$\Delta G$ +; not favored	$\Delta G$ +; not favored
-	+	$\Delta G$ -; favored	$\Delta G$ -; favored
-	-	$\Delta G$ -; favored	$\Delta G$ +; not favored

## Free Energy and Chemical Reactions

- If this were all there were to Free Energy, it would not be very useful. But we can use another approach to define free energy, which is particularly useful for studying chemical reactions.
- The chemical potential of compound A,  $\bar{G}_A$ , in solution is given by,  $\bar{G}_A = \bar{G}_A^\circ + RT \ln[A]$ , where  $\bar{G}_A^\circ$  is the free energy of A in its standard state and [A] is the actual molar concentration of A. A similar equation can be written for each component of the solution.
- For the reaction,  $aA + bB \rightleftharpoons cC + dD$ , the overall free energy change is given by  $\Delta G = c\bar{G}_C + d\bar{G}_D - a\bar{G}_A - b\bar{G}_B$ .
- After substituting the equations for the chemical potential of each component, we get 
$$\Delta G = \Delta G^\circ + RT \ln \frac{[C]^c [D]^d}{[A]^a [B]^b}$$
.
- $\Delta G^\circ$  is the free energy change for the reaction when each component starts out in its standard state.

Alternatively, we can determine  $\Delta G^\circ$  by noting that at equilibrium  $\Delta G = 0$ , which means that  $\Delta G^\circ = -RT \ln K_{eq}$ .

## Equilibrium Constants and Free Energy Changes

One of the important reactions in biochemistry is the hydrolysis of ATP



How do we calculate the free energy change for this reaction?

One of the important reactions in biochemistry is the hydrolysis of ATP



How do we calculate the free energy change for this reaction?

$$\Delta G = \Delta G^\circ + RT \ln \frac{[\text{ADP}^{-3}][\text{HPO}_4^{-2}][\text{H}^+]}{[\text{ATP}^{-4}][\text{H}_2\text{O}]}$$

- The free energy change for this reaction is
- In biochemistry, we make two simplifying assumptions in dealing with these problems:
  - The concentration of water does not change during the reaction.
  - The pH = 7.0 and does not change during the reaction.
- Under these conditions the [H<sub>2</sub>O] and [H<sup>+</sup>] are incorporated into ΔG<sup>o</sup> to give a new standard free energy change, ΔG<sup>o</sup>.
- Using these assumptions, and for simplicity, using
  - ADP instead of ADP<sup>-3</sup>
  - ATP, instead of ATP<sup>-4</sup>
  - P<sub>i</sub>, instead of HPO<sub>4</sub><sup>-2</sup>.

$$\Delta G = \Delta G^\circ + RT \ln \frac{[\text{ADP}][\text{P}_i]}{[\text{ATP}]}$$

- The free energy equation becomes

## Some Practical Considerations

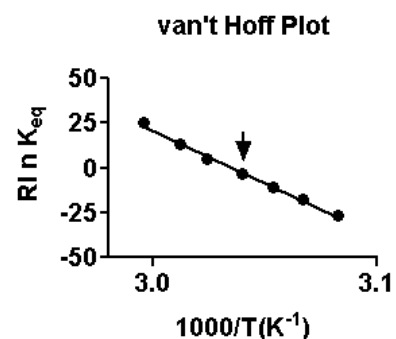
How do we obtain values for ΔH, ΔG and ΔS?

- One way is to study the temperature dependence of the equilibrium constant for a reaction. According to the van't Hoff equation

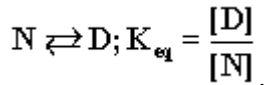
$$\Delta H^\circ = -R \frac{d(\ln K_{eq})}{d(1/T)}$$

. So, a plot of R ln K<sub>eq</sub> vs. 1/T (or 1000/T to keep the numbers greater than 1) should be a straight line with a slope = -ΔH<sup>o</sup>, the enthalpy change in the standard state.

- These data show the temperature dependence for the thermal denaturation of a protein, a process, which can



be described as the conversion from the native state N to the denatured state D -



- This plot has a slope of - 586 = -  $\Delta H^\circ$ , so  $\Delta H^\circ = 586$  kJ/mol - unfavorable.
- At 54.5°,  $K_{eq} = 0.27$ , so  $\Delta G^\circ = 3.56$  kJ/mol - unfavorable at this temperature.
- Recall that  $\Delta G = \Delta H - T\Delta S$ , so  $\Delta S^\circ = (\Delta H^\circ - \Delta G^\circ)/T = 1,778$  J/mol\*K - favorable.

## Sample Calculations

Now we can do some calculations

1. If the equilibrium concentrations of ATP =  $1 \times 10^{-7}$  M, ADP = 0.165 M and  $P_i = 0.1$  M, what is the equilibrium constant and  $\Delta G^\circ$  for the hydrolysis of ATP at 37°C?

- Use the data to calculate  $K_{eq}$

$$K_{eq} = \frac{[ADP][P_i]}{[ATP]}$$

$$K_{eq} = \frac{[0.165][0.10]}{[1 \times 10^{-7}]} = 1.65 \times 10^5$$

- Use the value of  $K_{eq}$  to calculate  $\Delta G^\circ$

- $\Delta G^\circ = -RT \ln K_{eq}$

- $\Delta G^\circ = -8.314 \times 10^{-3} \text{ kJ/mol} \times 310^\circ \times \ln(1.65 \times 10^5)$

- $\Delta G^\circ = -31$  kJ/mol.

2. In a typical cell at 37°C the concentration of ATP =  $8 \times 10^{-3}$  M, ADP =  $1 \times 10^{-3}$  M, and  $P_i = 8 \times 10^{-3}$  M. What is the free energy of ATP hydrolysis under these conditions?

$$\Delta G = \Delta G^\circ + RT \ln \frac{[ADP][P_i]}{[ATP]}$$

- $\Delta G = -31 \text{ kJ/mol} + RT \ln \{ [1 \times 10^{-3}][8 \times 10^{-3}] / [8 \times 10^{-3}] \} = -49 \text{ kJ/mol}$

## Coupled Reactions

Many of the processes that characterize life require the input of energy - they are **endergonic**

- An endergonic reaction is one with an unfavorable or positive free energy change.
- However, an endergonic reaction can be made to proceed in the desired direction if it is coupled to an **exergonic** reaction.
- An exergonic reaction is one with a favorable or negative free energy change.

For example, the phosphorylation of glucose to produce glucose-6-phosphate is a very important reaction in the cell.

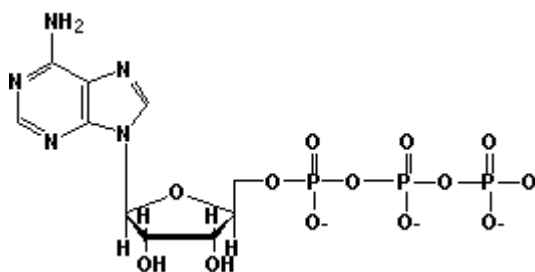
- **Glucose + P<sub>i</sub> ⇌ glucose-6-phosphate + H<sub>2</sub>O**,  $\Delta G^{\circ} = + 14 \text{ kJ/mol}$ , an unfavorable reaction!
- **ATP + H<sub>2</sub>O ⇌ ADP + P<sub>i</sub>**,  $\Delta G = - 31 \text{ kJ/mol}$ , a favorable reaction.
- By coupling the two reactions
  - **Glucose + P<sub>i</sub> ⇌ glucose-6-phosphate + H<sub>2</sub>O**  $\Delta G^{\circ} = + 14 \text{ kJ/mol}$
  - **ATP + H<sub>2</sub>O ⇌ ADP + P<sub>i</sub>**  $\Delta G^{\circ} = - 31 \text{ kJ/mol}$
  - -----
  - **Glucose + ATP ⇌ glucose-6-phosphate + ADP**  $\Delta G^{\circ} = - 17 \text{ kJ/mol}$
- Now we have an exergonic reaction, so the reaction can proceed, but at what rate?
- **A catalyst (the enzyme hexokinase) is required for the biologically appropriate reaction rate.**
- This is the strategy used in metabolic pathways.

## High-Energy Compounds

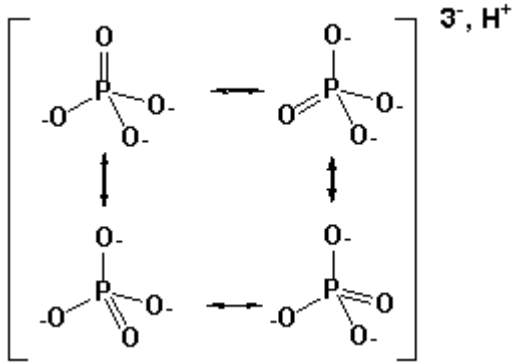
- The energy to drive endergonic reactions comes from the oxidation of foods- an exergonic process.
- During the oxidation of foods, the energy released is trapped in the form of a few energy-rich or "**high energy**" compounds, such as **ATP**.
- What is meant by "high energy" is that the free energy of transfer of the phosphoryl group from the phosphoanhydride of ATP to another compound proceeds with a large negative  $\Delta G$ .
  - If this transfer is to water, then it is hydrolysis, where  $\Delta G^{\circ} = -31 \text{ kJ/mol}$
  - The hydrolysis of an ordinary phosphodiester bond has a  $\Delta G^{\circ} = -14 \text{ kJ/mol}$ .
- As shown above, this free energy of phosphoryl transfer can be used in coupled reactions to drive unfavorable reactions.

## ATP

What makes the phosphoanhydride bond of ATP energy-rich? Why is ATP so important as the energy currency of the cell?



The charged phosphate groups in ATP are forced into close proximity. Some of this unfavorable electrostatic interaction is relieved when ATP is converted to ADP; an equal amount is relieved when ADP is converted to AMP - the hydrolysis of ADP also has a  $\Delta G^{\circ} = - 31 \text{ kJ/mol}$ .



The phosphate ion can have several different resonance forms, which gives it a high entropy. The phosphate group has fewer resonance forms in ATP. Therefore the hydrolysis of ATP frees the phosphate ion, which is an entropically favorable process.

## The Importance of Metastability

A **metastable** compound is thermodynamically unstable, but in the absence of a catalyst will break down only slowly - ATP is thermodynamically unstable but kinetically stable. Thus, the energy stored in the phosphoanhydride bond is not squandered by random hydrolysis but its energy is released only under controlled (catalyzed) conditions.

## Ligand Binding

A common event in biological systems is the noncovalent binding of one or more small molecules (**ligand**) to a macromolecule, such as a protein. In fact, the biological activity of most proteins, for example enzymes, is dependent on such binding. Analysis of binding equilibria requires a few simple algebraic equations.

- We begin by writing the equation for the **dissociation** of the ligand (L) from the protein (P)  $P \cdot L \rightleftharpoons P + L$

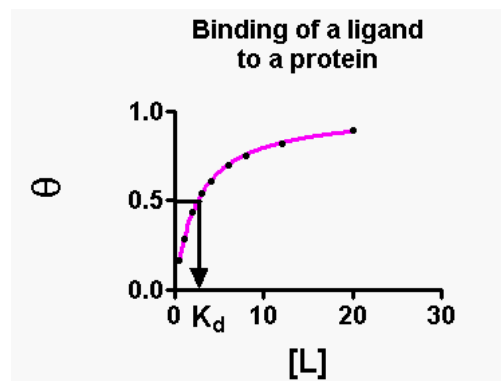
$$K_d = \frac{[P][L]}{[PL]}$$

- Next we write the dissociation constant for the reaction

$$\theta = \frac{[L]_{\text{bound}}}{[P]_{\text{total}}} = \frac{[PL]}{[P] + [PL]}$$

- A useful parameter for plotting data is  $\theta$ , where

$$\theta = \frac{[L]}{K_d + [L]}$$



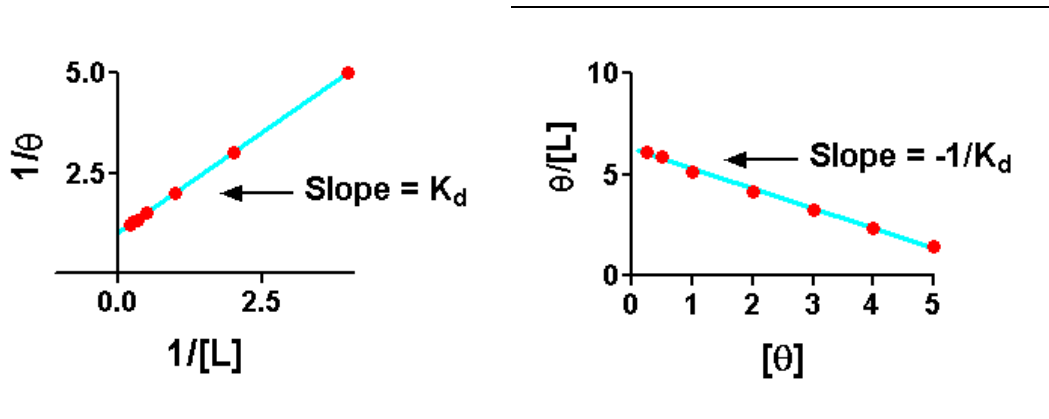
Combining equation 2 and 3 gives  $\theta = \frac{[L]}{K_d + [L]}$ . This is the equation of a **hyperbola**. The concentration of the ligand, [L], for which  $\theta = 0.5$  is equal to the  $K_d$ . It is, however, difficult to accurately estimate  $\theta = 0.5$ , because the saturation curve approaches  $\theta = 1.0$  asymptotically.

$$\frac{1}{\theta} = \frac{K_d}{[L]} + 1$$

Usually, a linear transformation of the binding equation is used, for example,

$$\frac{\theta}{[L]} = \frac{1}{K_d} - \frac{\theta}{K_d}$$

or  $\frac{\theta}{[L]} = \frac{1}{K_d} - \frac{\theta}{K_d}$ . Such equations give linear plots, which makes determination of  $K_d$  more accurate.



- Once  $K_d$  has been determined, then the free energy of dissociation can be determined from  $\Delta G^\circ = -RT \ln K_d$ . The free energy of association or **the binding free energy is  $-\Delta G^\circ$  of dissociation, because  $K_a = 1/K_d$ .**

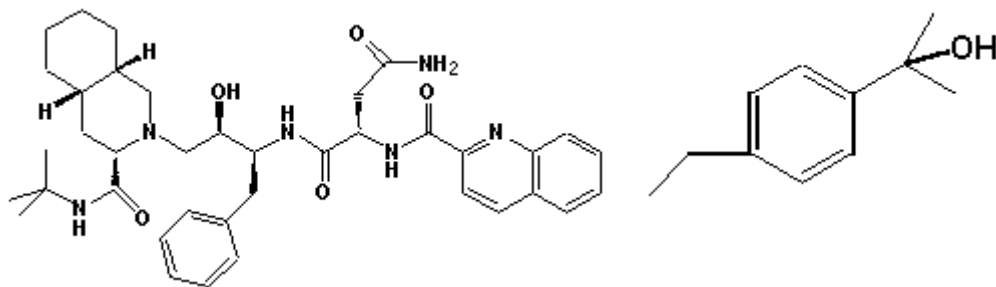
### Example

For the binding of a certain ligand to a protein at  $25^\circ \text{C}$  and  $\text{pH}=7.0$ ,  $K_d = 1 \times 10^{-7} \text{ M}$ . What is the free energy of association of the ligand with the protein?

- For the dissociation reaction,  $\Delta G^\circ = -RT \ln K_d$
- $\Delta G^\circ = -(8.314)(298)(-16.1) = + 39.9 \text{ kJ/mol}$  - **Dissociation is not favored.**
- For the association reaction,  $\Delta G^\circ = -RT \ln K_a = -RT \ln(1/K_d)$ .
- $\Delta G^\circ = -(8.314)(298)(16.1) = - 39.9 \text{ kJ/mol}$  - **Association is favored.**

### Thermodynamics and the HIV virus

- During replication, the AIDS virus produces a large polypeptide chain, which is cleaved into smaller proteins required for the assembly of the virus.
- The cleavage of the precursor protein is carried out by a specific enzyme called the HIV protease.
- In the absence of the HIV protease, the virus can not mature and can not infect cells.
- Therefore, the HIV protease has been the target of intense study to try to discover specific inhibitors that can prevent virus replication.
- A variety of different compounds have been developed, as shown below.



These compounds all have high affinity for the HIV protease,  $K_d = 5-15 \times 10^{-8} \text{ M}$  .

### The virus fights back!

- The presence of the inhibitor places strong selective pressure on the virus to evolve an inhibitor-resistant form of the protease. Apparently, the virus is more concerned with having a protein with a low affinity for the inhibitor than with a protein with high enzymatic activity.
- Binding of inhibitors to the wild type HIV protease and some resistant mutants.

Compound	$K_d(\text{nM})$			
	Wild Type	V82F	I84V	V82F/I84V
1	0.15	0.50	0.25	2.85
2	0.17	0.14	1.90	120.0
3	0.08	0.11	1.70	120.0
4	0.08	0.03	0.20	3.36
<b>% Activity</b>	<b>100</b>	<b>42</b>	<b>75</b>	<b>11</b>

- How much of a change in  $K_d$  is necessary to significantly alter the amount of inhibitor bound?

