MICROBIOLOGY COURSE MATERIAL

CC3: Thermodynamics

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CC-3: Biochemistry (THEORY)
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SYLLABUS: UNIT 1: BIOENERGETICS

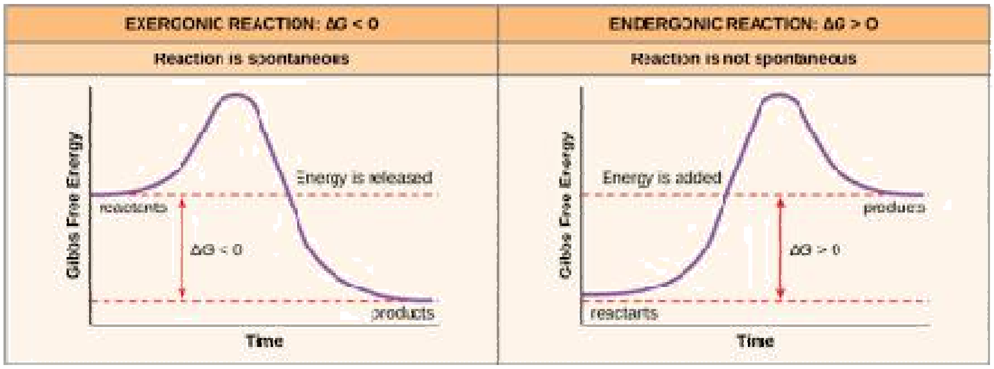
- First and second laws of Thermodynamics.
- Definitions of Gibb's Free Energy, enthalpy, and Entropy and mathematical relationship among them.
- Standard free energy change and equilibrium constant Coupled reactions and additive nature of standard free energy change.
- Energy rich compounds: Phosphoenolpyruvate, 1, 3-Bisphosphoglycerate, Thioesters, ATP

❖ Standard Free Energy Change and Equilibrium Constant

➢ THERMODYNAMICS

Thermodynamics is the branch of physical chemistry that deals with the energy changes. And biochemical thermodynamics (or biochemical energetics or bioenergetics, as it is also called) is the field of biochemistry concerned with the transformation and use of energy by living cells. The chemical reactions occurring in living beings (or biochemical reactions) are associated with the liberation of energy as the reacting system moves from a higher to a lower energy level. Most often, the energy is liberated in the form of heat. In non-biological systems, heat energy may be transformed into mechanical or electrical energy. Since the biologic systems are isothermic, the heat energy cannot be used to drive the vital processes (such as synthesis, active transport, nerve conduction, muscular contraction etc.) **obtain energy by chemical linkage (or coupling) to oxidation reactions.** The simplest type of coupling may be represented by the equation.

$$\mathbf{A + C \rightarrow B + D + H}$$



The conversion of metabolite A to metabolite B occurs with the release of energy. It is coupled to another reaction, wherein energy is required to convert metabolite C to metabolite D.

- **THERMODYNAMIC PRINCIPLES**

Many quantitative observations made on the interconversion of various forms of energy have led scientists to the formulation of two fundamental laws of thermodynamics, the first and second. These laws help us understanding:

1) the direction of a reaction, whether from left to right or vice versa,

2) the accomplishment of work, whether useful or not, and

3) whether the energy for driving a reaction must be delivered from an external source.

- **The First Law**:

Principle of Conservation of Energy In thermodynamics, a system is a matter within a defined region. The matter in the rest of the universe is called the surroundings. Thus, the system plus the surroundings constitute the universe which literally includes the entire earth, rather even the outer space.

In the biological world, the reacting system do exchange energy, and often matter also with their surroundings.

The first law of thermodynamics states that the total amount of energy in the universe (i.e., the system + surroundings) remains constant. Paraphrased, it says that energy cannot be created or destroyed. To date, there is no known exception to this law. Thus, whenever energy is used to do work or is converted from one to the other form, the total amount of energy is unchanged.

The mathematical expression of the first law is:

$$\Delta E = EB - EA = Q - W \ldots (1)$$

where, ΔE = change in internal energy

Δ EA = energy of a system at the start of a process

Δ EB = energy of a system at the end of a process

Δ Q = heat absorbed by the system

W = work done by the system.

A noteworthy point about equation 1 is that the change in energy of a system depends only on the initial and the final stages and not on the path of transformation.

- **The Second Law**

The first law of thermodynamics cannot be used to predict whether a reaction can occur spontaneously, although Δ E is positive. In such cases, the system absorbs heat from its surroundings so that the sum of the energies of the system and its surroundings remains the constant. It is evident that a function other than Δ E is required. One such function is entropy, which is denoted by the symbol S. Entropy is energy in a state of randomness or disorder. It is unavailable, useless energy. **In other words,** entropy is a measure of the degree of randomness or disorder of a system. The entropy of a system increases (i.e., Δ S is positive) when it becomes more disordered. Entropy becomes maximum in a system as it approaches equilibrium. When equilibrium is attained, no further change can occur spontaneously unless additional energy is supplied from outside the system.

Thus, according to the second law, at each transfer or transformation of energy, part of that energy assumes a form that cannot be passed on any further because it is randomly dispersed, often as heat. And it is this randomly dispersed energy which is called entropy. Ultimately closed systems run down. Open systems, such as ecosystems, however, with their constant input and outflow, maintain a steady state in spite of the second law of thermodynamics. Biological systems, thus, do not seem to conform to the second law, for the tendency of life is to produce order out of disorder, to decrease rather than increase entropy.

The second law of thermodynamics states that a process can occur spontaneously only if the sum of the entropies of the system and its surroundings increases. This can be represented as:

(ΔSsystem + ΔSsurroundings) > 0 for a spontaneous process … (2)

Thus, the total entropy of a system must increase if a process is to occur spontaneously. However, the entropy of a system can decrease even during a spontaneous process, provided the entropy of the surroundings increases to such extent that their sum is positive.

When water freezes, its entropy decreases because the water molecules of ice exist in a more ordered state with less freedom of movement than in a liquid state. The decrease of entropy is particularly apparent in the formation of a snowflake.

The difference between the first and second laws of thermodynamics is that the first law is concerned with the accounting of the various kinds of energy involved in a given process, whereas the second law is concerned with the availability of the energy of a given system for doing useful work.

Combining the Two Laws since the entropy changes of chemical reactions are not readily measurable, the entropy is not used as a criterion whether a biochemical process can occur spontaneously or not. Furthermore, for spontaneity, both the entropy changes (that of the surroundings and system) should be known. These difficulties are alleviated by using a different thermodynamic function, called the free energy, which is denoted by the symbol G (In older literature, however, the symbol F is used in its place).

In 1878, Gibbs created the free energy function by combining the first and second laws of thermodynamics in the form of following equation:

$$\Delta G = \Delta H - T\Delta S \quad \dots (3)$$

where,

ΔG = the change in free energy of a reacting system,

ΔH = the change in heat content or enthalpy of this system,

T = the absolute temperature at which the process is taking place, and

ΔS = the change in entropy of this system.

In fact, this equation represents quantitative interrelationship between the changes in free energy, heat and entropy in chemical reactions at constant temperature (T) and pressure (P), the conditions prevailing in biological systems. The term $T\Delta S$ is that fraction of ΔH which cannot be put to useful work. **The ΔG indicates the free energy change or the theoretically available useful work.** Naturally, in most cases the system is "inefficient" and not all of the theoretically available work can be utilized. However, the properties of the surroundings do not enter into this equation.

The enthalpy change, ΔH is given by the following equation:

$$\Delta H = \Delta E + P\Delta V \qquad \dots (4)$$

where,

ΔE = the change in internal energy of a reaction, and

ΔV = the change in volume of this reaction.

As the volume change, ΔV is small for nearly all biochemical reactions, hence ΔH is nearly equal to the change in internal energy, ΔE. Therefore, equation 3 modifies as follows:

$$\Delta G \cong \Delta E - T\Delta S \quad ...(5)$$

Thus, the change in free energy of a reaction**,** ΔG depends both on the change in internal energy and on the change in entropy of the system. The ΔG is a valuable criterion in determining whether a reaction can occur spontaneously. Thus,

(a) **If ΔG is negative in sign, the reaction proceeds spontaneously with loss of free energy, i.e., it is exergonic**. If, in addition, ΔG is of great magnitude, the reaction goes virtually to completion and is essentially irreversible.

(b) **If, however, ΔG is positive, the reaction proceeds only if free energy can be gained, i.e., it is endergonic**. If, in addition, ΔG is of high magnitude, the system is stable with little or no tendency for a reaction to occur.

(c**) If ΔG is zero, the reaction system is at equilibrium and no net change takes place.** With regard to the free energy change, ΔG of a reacting system two more points need to be emphasized:

I. Firstly, the ΔG of a reaction depends only on the free energy of the products minus that of the reactants. The ΔG of a reaction is independent of the path of transformation. Obviously, the mechanism of a reaction has no effect on ΔG.

As an instance, the value of ΔG is the same for the oxidation of glucose to CO_2 and H_2O whether it takes place by combustion or by a series of enzyme-catalyzed reactions.

II. Secondly, the value of ΔG provides no information about the rate of a reaction. A negative ΔG indicates that a reaction can occur spontaneously, but it does not signify that it will occur at a perceptible rate. As already pointed out, the rate of a reaction rather depends on the free energy of activation ($\Delta G^{\neq}$), which is unrelated to ΔG. To get a feeling for the magnitude of changes in various forms of energy, an actual example of aerobic oxidation of glucose may be cited.

The living cells carry out oxidation of glucose in the presence of oxygen to CO_2 and H_2O at constant temperature and pressure.

$$\mathbf{C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O}$$

Assuming that the temperature is 25°C (or 298 K) and the pressure is 1.0 atm. (or 760 mm Hg), which are standard conditions in thermodynamic calculations, the following energy changes take place per molecule of glucose oxidized :

$\Delta G = -\ 686{,}000$ cal/mol (i.e., the free energy of the reacting molecules has decreased)

$\Delta H = -\ 673{,}000$ cal/mol (i.e., the reacting molecules have released heat)

The equation 3 may also be written as:

$$\mathbf{\Delta S = \Delta -\Delta\ H\ G\ T\ \ldots(6)}$$

Substituting the above values in equation 6, we get : $\Delta S = 673{,}000\ (\ 686{,}000)\ 298\ --\ = 44$ cal/deg (i.e., the entropy of the universe has increased)

❖ RELATIONSHIP BETWEEN STANDARD FREE ENERGY CHANGE AND EQUILIBRIUM CONSTANT

In a model reaction, **$A + B \leftrightarrow C + D$ ...(7)**

The free energy change, ΔG of this reaction is given by

$\Delta G = \Delta G° + RT \ln[C][D] / [A][B]$...(8) where,

$\Delta G°$ = Standard free energy change,

R = Gas constant,

T = Absolute temperature,

and [A], [B], [C] and [D] = Molar concentrations (i.e., activities) of the reactants To elaborate, ΔG° is the free energy change for this reaction under standard conditions, i.e., when each of the reactants A, B, C and D is present at a concentration of 1.0 M. Thus, the ΔG of a reaction depends on the nature of the reactants (expressed in ΔG° term) and on their concentrations (expressed in logarithmic terms), as shown in equation 8. At equilibrium, ΔG = 0. Equation 8 then becomes

$$O = \Delta G° + RT \ln [C][D] / [A][B] \text{...(9)}$$

$$\text{or } \Delta G° = -RT \ln[C][D] / [A][B] \text{ ...(10)}$$

The equilibrium constant under standard conditions, K′ eq for the reaction

$A + B \leftrightarrow C + D$, is given by $K'_{eq} = [C][D] / [A][B]$...(11)

However, in reactions in which more than one molecule of any reactant or product participates, the general reaction— aA + bB ↔cC + dD where a, b, c and d are the number of molecules of A, B, C and D participating, the equilibrium constant is given by $K'eq = [C]^c[D]^d / [A]^a[B]^b$...(12)

Substituting equation 11 into equation 10 gives -

$$\Delta G° = -RT \ln K'eq \ ...(13)$$

or $\Delta G° = -2.303\ RT \log_{10} K'eq$ ...(14) Substituting R = 1.98 × 10–3 kcal mol–1 degree–1 and T = 298°K (corresponding to 25°C) gives the standard free energy, ΔG°and the equilibrium constant, K′ eq are related by a simple expression**. For example, a change in equilibrium constant by a factor of 10 results in a change in standard free energy of –1.36 kcal/mol at 25°C** at 37°C, however, the change in standard free energy would be of 1.42 kcal/mol. Values of ΔG° may be expressed in joules or calories per mole

ΔG° may also be defined as the difference between the free energy content of the reactants and that of the products under standard conditions (i.e., 298 K pressure and 1.0 atmospheric pressure) when the reactants and products are present in their standard concentrations, namely 1.0 M.

When ΔG° is negative (i.e., the products contain less free energy than the reactants), the reaction will proceed to form the products under standard conditions, since all chemical reactions will tend to go in that direction resulting in decrease in the free energy of the system. When ΔG° is positive (i.e., the products of the reaction contain more free energy than the reactants), the reaction will tend to go in the reverse direction if we start with 1 M concentration of all components.

To be more explicit, reactions with a negative ΔG° proceed forward in the direction written when they start with all reactants and products at 1.0 M, until they reach equilibrium. Reactions with a positive ΔG° will proceed in the reverse of the direction written when they start with all components at 1.0 M. Following Table summarizes these relationships.

Relationship among K′eq, ΔG° and the direction of chemical reactions under standard conditions When K′ eq is : ΔG° is : Starting with 1 M components, the reaction: > 1.0 Negative proceeds forward. 1.0 Zero remains at equilibrium, < 1.0 positive proceeds backward. Two more points need be emphasized:

I. Biochemical reactions take place near pH 7.0. Hence, **pH 7.0 is conventionally designated the standard pH in biochemical** energetics. The standard free energy change at pH 7.0 in biochemical energetics is designated by the symbol, Δ**G°′,** which shall be used in further discussions.

II. The basic SI (Systéme International) unit of energy is joule (J). However, in biology and medicine, heat and energy measurements are equally expressed in calories (cal).

The interrelationship between colories and joules is: 1.000 cal = 4.184 J **STANDARD FREE ENERGY CHANGES AT pH 7.0 OR ΔG°′**

To calculate ΔG°′, an example of isomerization of dihydroxyacetone phosphate (DHAP) to glyceraldehyde 3-phosphate (G-3-P) may be taken. This is one of the reactions of glycolysis. James Prescott Joule was a British engineer who experimentally proved the first law of thermodynamics. He determined the mechanical equivalent of heat in 1843. The unit Joule is named after him. A Joule

(J) is the amount of energy required to apply a 1 Newton force over a distance of 1 metre. A kilo Joule (kJ) is equal to 1,000 J.

A calorie (cal) is equivalent to the amount of heat needed to raise the temperature of 1 gram of water from 14.5 to 15.5°C.

A kilocalorie (kcal) is equal to 1,000 cal. Also, 1 kcal = 4.184 kJ.

At equilibrium, the ratio of glyceraldehyde 3-phosphate to dilydroxyacetone phosphate is 0.0475 at 25°C (298°K) and pH 7.0. Hence, $K' eq = 0.0475$
The standard free energy change for this reaction is, then, calculated, as follows:

$\Delta G° = –2.303\ RT \log_{10} K'\ eq = –2.303 \times 1.98 \times 10–3 \times 298 \times \log_{10} (0.0475) = +1.8$ k cal/mol When the initial concentration of DHAP is 2×10^{-4} M and the initial concentration of G-3- P is 3×10^{-6} M, the ΔG can be calculated from above equation, as follows : $\Delta G = \Delta G° + RT \log_e [C][D] / [A][B]$

= = –0.7 kcal/mol

The negative value for ΔG points out that isomerization of dihydroxyacetone phosphate to glyceraldehyde 3-phosphate can occur spontaneously, when these compounds are present at concentrations mentioned above. Although ΔG is negative for this reaction, the value of ΔG°′ is positive. It is important to note that the magnitude of ΔG for a reaction (whether smaller, larger or the same as ΔG°′) depends on the concentrations of the reactants .The criterion of spontaneity for a reaction is ΔG and not ΔG°′.

➢ Difference Between ΔG and ΔG°′

The difference between the free energy change, ΔG and the standard free energy change, ΔG°′ of a chemical reaction should be clearly understood. The actual free energy change, ΔG of a chemical reaction is a function of the conditions of concentration, pH and temperature under which the reaction is taking place. Moreover, the ΔG of an ongoing chemical process is always negative, becomes smaller (i.e., less negative) as the reaction proceeds and is zero at the point of equilibrium, indicating that no more work can be done by the reaction. The value of ΔG declines with time as the reaction proceeds because the actual concentrations of the reactants will be getting smaller and those of the resultants getting larger.

Thus, in every spontaneous chemical or physical process, the free energy of the reacting system always decreases, i.e., ΔG is negative. On the contrary, the value of standard free energy change, ΔG°′ for a chemical reaction is characteristic and unchanging, and may be positive, negative or zero, depending on the equilibrium constant of the reaction. ΔG°′ is, thus, an immutable constant and tells us in which direction and how far a given reaction will go in order to reach equilibrium when it occurs under standard conditions, i.e., when the initial concentration of all components is 1.0 M, pH is 7.0 and the temperature is 25°C

➢ Biomolecular Interactions

All functions of living systems ranging from primitive bacteria to higher-order organisms, as well as their interactions with their environment, are realized through macromolecular interactions. These interactions might be simple or rather complex— with at least one of the partners being a biological macromolecule, usually a protein. In this chapter we briefly discuss the various types of interactions of proteins, their molecular background, theory and some practical applications.

Protein interactions can be classified into different groups regarding the molecular properties and functions of the interacting partners. (These groups are intertwined in several cases.) Some examples include:

A. The interactions of proteins with other proteins, small molecules, carbohydrates, lipids or nucleic acids;
B. Receptor-ligand interactions;
C. Antigen-antibody interactions;
D. Enzymatic interactions, enzyme-inhibitor interactions.

In the following, these reactions will be denoted as protein-ligand interactions. Such interactions often play key roles in the initiation of complex multi-step reactions. As an example, ligand binding—such as that of steroid hormones to their cytoplasmic or nuclear receptors or the binding of secreted peptide ligands to transmembrane receptors—might activate a receptor molecule, which induces a cellular response.

➢ **Reaction Kinetics**

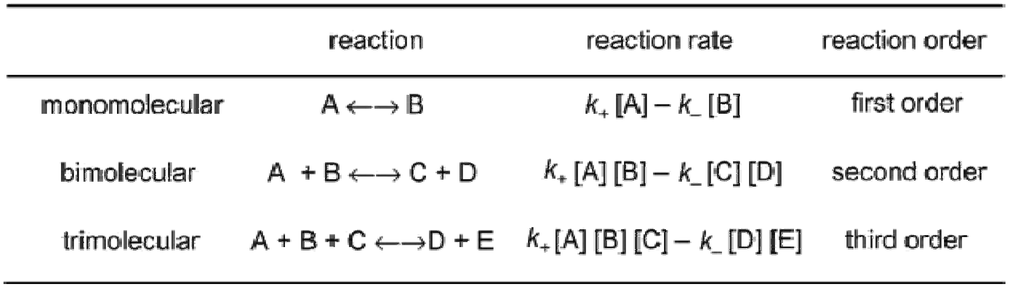
Factors that determine a particular protein-ligand interaction include the concentrations of the partners, the binding affinity, and the rate constants of association and dissociation. As a theoretical introduction, we will first provide a brief and simplified description of reaction kinetics. Let us consider the following interaction:

$$mA + nB \underset{k_-}{\overset{k_+}{\rightleftharpoons}} C + D \qquad \textbf{(1)}$$

The reaction rate will be the sum of the rates of product formation and the rate of the reverse reaction:

$$v = k_+ [A]^m [B]^n - k_- [C][D] \qquad \textbf{... (2)}$$

Where, k_+ and k_- are the rate constants, m is the order of reaction with respect to A, n is the reaction order for B, and m+n provides the order of the overall reaction. A few simple general reactions are listed in Figure 1.



	reaction	reaction rate	reaction order
monomolecular	$A \longleftrightarrow B$	$k_+ [A] - k_- [B]$	first order
bimolecular	$A + B \longleftrightarrow C + D$	$k_+ [A] [B] - k_- [C] [D]$	second order
trimolecular	$A + B + C \longleftrightarrow D + E$	$k_+ [A] [B] [C] - k_- [D] [E]$	third order

Figure 1: Examples for reaction rates and reaction orders

In equilibrium, the overall reaction rate is zero, i.e. the rates of product formation and dissociation are equal and the concentrations of the reactants and products are constant, having equilibrium values:

$$k_+ [A]^m [B]^n = k_- [C][D] \qquad \textbf{... (3)}$$

The equilibrium dissociation (and association) constants can be defined as the ratio of the products of concentrations of the reacting molecules and the products of the concentrations of the resulting molecules, with the concentrations raised to the power reflecting the stoichiometry of the components:

$$K_D = k_- / k_+ = ([A]^m[B]^n) / ([C][D]) \qquad \textbf{... (4)}$$

The association constant is the reciprocal of the dissociation constant:

$$K_A = 1 / K_D \qquad \text{... (5)}$$

The equilibrium association and dissociation constants describe the extent to which the reaction is shifted towards the formation of the products. In the case of protein-ligand interactions (see below), these characterize the binding affinity between the components.

Far from the equilibrium state, the —on or the —off rate can be dominating. In such cases, the rate constants can be determined directly by rapid kinetic experiments.

We want to answer these questions:

- Will this reaction go?
- If so, how far can it proceed? We will do this by using thermodynamics

- **Protein-ligand interactions**

After the general discussion, we will take a simple second-order protein-ligand interaction model. From now on, we will denote the reactants by their initials, *P* and *L*, and their protein-ligand complex as *PL*:

$$\text{Protein} + \text{Ligand} \Leftrightarrow \text{Protein-Ligand Complex} \qquad \text{... (6)}$$

$$\mathrm{P} + \mathrm{L} \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} \mathrm{PL}$$

Where, k_{on} and k_{off} are the rate constants of binding and dissociation, respectively.

The equilibrium association and dissociation constants are as follows:

$$K_A = \frac{[\mathrm{PL}]}{[\mathrm{P}][\mathrm{L}]} = \frac{k_{on}}{k_{off}} \quad \textbf{... (7)}$$

$$K_D = \frac{[\mathrm{P}][\mathrm{L}]}{[\mathrm{PL}]} = \frac{k_{off}}{k_{on}} \quad \textbf{... (8)}$$

Where, [P] is the concentration of free ligand molecules and [PL] is the concentration of the protein-ligand complex. In this simple reaction, the unit of the dissociation constant is concentration (M, mol/litre). The lower the dissociation constant, the stronger is the binding. In biological systems, ―tight binding‖ corresponds to a dissociation constant in the order of 10^{-9} M (nM) or less. In some high-affinity enzyme-inhibitor interactions, such as the formation of the bovine trypsin inhibitor (BPTI)-trypsin complex, the K_D value is in the order of 10^{-13} M (sub-picomolar).

- **Relationship between the free enthalpy (Gibbs free energy) change and the equilibrium constant**

As a consequence of the second law of thermodynamics, at constant pressure and temperature, a reaction will spontaneously take place if the Gibbs free energy (free enthalpy) difference between the final state and the initial state is negative, i.e. if $G < 0$. During the approach to equilibrium, the free enthalpy decreases, reaching a minimum at equilibrium where $G = 0$. As a reminder, the definition of the Gibbs free energy is:

$$G = H - T S \quad \textbf{... (9)}$$

Where, H is the enthalpy, T is the absolute temperature and S is the entropy. In solutions, the standard free enthalpy, G°, is defined for a standard state in which the concentration of each component is 1 M. With respect to this reference value, the free enthalpy of the system of a solution of molecule *A* can be expressed in the form:

$$G = G^0 + RT \ln a \qquad \textbf{... (10)}$$

Where, *a* is the activity (effective concentration), *R* is the universal gas constant, which has the value 8.31447 $J{\cdot}K^{-1}{\cdot}mol^{-1}$. In dilute solutions, *a* approximately equals the molar concentration of molecule *A* ([*A*]). Therefore:

$$G = G^0 + RT \ln [A] \qquad \textbf{... (11)}$$

In solution phase, in the case of a reaction with several components, the free enthalpy change of the reaction can be expressed as:

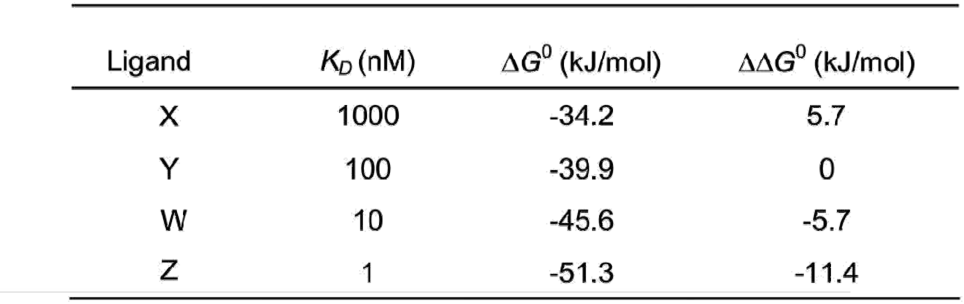
$$\Delta G = \Delta G^0 + RT \ln \frac{\prod[\text{products}]}{\prod[\text{reactants}]} \qquad \textbf{... (12)}$$

Where, in the fraction, the products of the concentrations appear. In equilibrium, *G* = 0 and the concentrations in the fraction are the equilibrium concentrations. Thus, the fraction gives exactly the equilibrium association constant. Therefore, the standard free enthalpy change can be determined directly from the equilibrium constant:

$$\Delta G^0 = - RT \ln K_A = RT \ln K_D \qquad \textbf{... (13)}$$

It is worth noting that, in biochemical systems, we use aqueous solutions in which the standard state is defined at neutral pH (pH 7.0).

G° describes the stability of the products in a reaction, or the stability of the complex in a protein-ligand interaction in the equilibrium, relative to the reactants. Figure 2 shows the relationship between the dissociation constant and $G°$ for different binding affinities, compared to the case at K_D = 100 nM (second line).



Ligand	K_D (nM)	ΔG^0 (kJ/mol)	$\Delta\Delta G^0$ (kJ/mol)
X	1000	-34.2	5.7
Y	100	-39.9	0
W	10	-45.6	-5.7
Z	1	-51.3	-11.4

Figure 2: Comparison of $G°$ at different binding affinities at 25°C