1st Year Thermodynamic Lectures
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Basic chemical thermodynamics :-

Lecture 1. Introduction. Basic Definitions. 1st Law of thermodynamics.

Lecture 2. 2nd Law and entropy. Free energy and equilibria. Single component systems.

Lecture 3. Chemical potential and multiple component systems.

Lecture 4. Determining thermodynamic functions. Multiple step processes.

BIBLIOGRAPHY

Principles and Problems in Physical Chemistry for Biochemists, 3rd edition, N.C. Price, R.A. Dwek, R.G. Ratcliffe and M.R. Wormald, Oxford University Press -- the standard 'Oxford 1st year biochemists' text.

Basic Chemical Thermodynamics, E.B. Smith, Oxford Chemistry Series, Oxford University Press -- the standard 'Oxford 1st year chemists' text on thermodynamics -- slightly more advanced and very good.

Physical Chemsitry for the Biomedical Sciences, S.R. Logan, Taylor & Francis.

Physical Chemistry for the Life Sciences, P.W. Atkins and J. de Paula, Oxford University Press -- more simple version of the ubiquitous 'Physical Chemistry' -- highly recommended.

Bioenergetics at a glance, D.A. Harris, Blackwell -- 1st few chapters give a good overview.

CONSTRAINTS IMPOSED ON BIOLOGICAL SYSTEMS BY PHYSICS AND CHEMISTRY

In order to exist, a living species has to:

1. collect energy from the surroundings
2. convert it to a useful form (ion gradients, ATP, etc)
3. use it to grow, change, replicate, etc.

Living systems can only do something if the energy needed is balanced by the energy that is available.

THERMODYNAMICS

"The Study of Heat and Work"

Thermodynamics allows us to investigate the distribution of energy in a system.

By comparing two systems, we can determine how much energy is needed to convert, or given out by converting, one systems to another.

We can apply thermodynamics to stable systems, unstable systems, or to each stage of a reaction mechanism.

Thermodynamics cannot give information on reaction rates directly (although the amount of energy required is going to be important in determining reaction rates - see Transition State Theory).

FEATURES

1. It is an empirical model -- we use it because it works.
2. It is based on a few initial postulates, the Laws of Thermodynamics, everything else follows logically.
3. It makes no assumptions about the nature of a system -- we do not even have to know whether or not molecules exist.

STANDARD STATES/CONDITIONS

As we are mostly comparing systems, it is useful to have constant reference points (standard states) against which all comparisons can be made.

Standard states for compounds are defined as :

Solid	pure solid
Liquid	pure liquid
Solute	concentration of 1 mol per kg of solvent in a given solvent
Gas	pure gas at 1 atm pressure

Standard conditions for all reactions of practical interest are defined as 1 atm pressure and 298 K.

Biochemical standard conditions are those in which all components are present in their standard states, except H^+ which is present at 10^{-7} mol dm^{-3}, i.e. pH = 7.0.

TYPES OF SYSTEMS

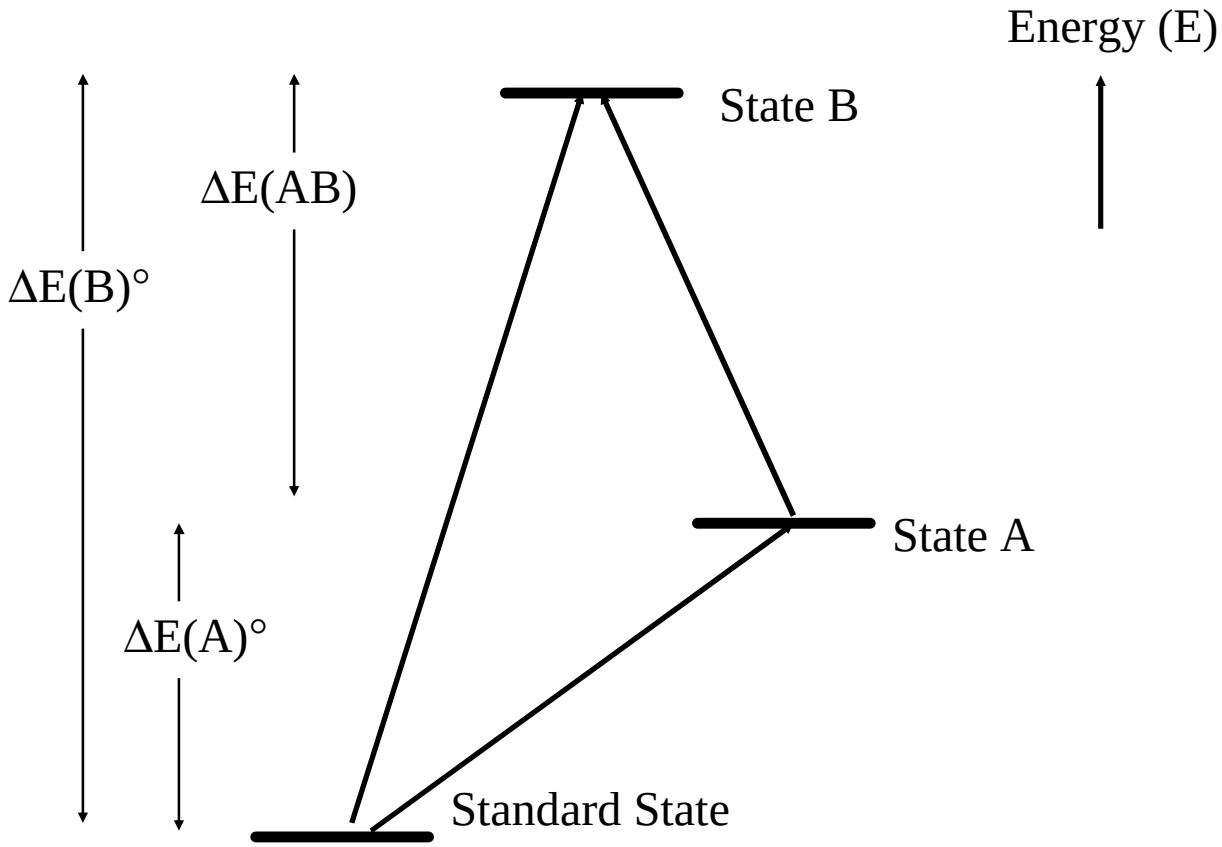
Open -- systems that can exchange matter and energy with the surroundings

Closed -- systems that can only exchange energy with the surroundings. Almost all chemical reactions fall into this category.

Isolated -- systems that cannot exchange either energy or matter with the surroundings. The most obvious case of an isolate system is the universe.

STATE FUNCTIONS

A state function depends only on the state of the system, not on the path taken to reach that state.



Intensive -- the property is independent of the size of the system (it has the same value for a small region of the system as for the whole system). e.g. temperature, pressure.

Extensive -- the property is dependent on the size of the system (the value changes between a small region of the system and the whole system). e.g. mass, volume, energy.

Most thermodynamic quantities are extensive state functions.

1st LAW OF THERMODYNAMICS
(The Law of Conservation of Energy)

The algebraic sum of all energy changes in an isolated system is zero.

We define U as the internal energy of a system. From the 1st Law, U must be a state function. Thus;

$$\Delta U = U_{final} - U_{initial}$$

For an isolated system, from the 1st Law $\Delta U = 0$.

For a closed system, the internal energy of the system can increase as long as the internal energy of the surroundings decrease by the same amount.

$$\Delta U_{system} = - \Delta U_{surroundings}$$

Work and heat :-

As we shall see, it is useful to distinguish between two classes of energy, work and heat. Again, we can define;

q - heat absorbed by the system from the surroundings
w - work done on the system by the surroundings

$$\Delta U_{system} = q + w$$

Distinction between work and heat :-

Work is the energy associated with orderly movements of bodies, for example pushing back boundaries (volume change) or moving electrons along a wire.

Heat is the energy associated with disorderly movements of bodies, for example random molecular motion in a gas.

Work in chemical systems :-

The work energy term (w) is also the sum of many different types of energy, the most common being electrical work (such as electrochemical cells), mechanical work (such as muscle contraction) and change of volume (expansion).

$$w = w_{electrical} + w_{mechanical} + w_{expansion}$$

Most of these are usually zero. However, at constant pressure (where life exists) the volume of the system often changes and thus does work by pushing back the surroundings. In this case, w is given by

$$w_{expansion} = - P\Delta V$$

ΔV is the increase in volume of the system. w is negative because, by increasing in volume, the system is doing work on the surroundings.

ENTHALPY

It is usually convenient to factor out the changes in internal energy due to changes in volume (ie. allow for constant pressure). Thus;

Change in internal energy allowing for volume changes = $\Delta U - w_{expansion}$

$$= \Delta U + P\Delta V$$

$$= (U_{final} + PV_{final}) - (U_{initial} + PV_{initial})$$

(U + PV) is an extensive state function and is called, for convenience, ENTHALPY, H.

$$H = U + PV$$

ΔH is a measure of the change in the total energy of a system after allowing for changes in volume.

For most chemical systems, the only other form of energy that is exchanged with the surroundings is heat. ΔH is then simply the heat absorbed at constant pressure.

DIRECTION OF A CHEMICAL REACTION

Dissolution of $NaNO_3$	Spontaneous	Endothermic (ΔH = +ve)
Dissolution of NaOH	Spontaneous	Exothermic (ΔH = -ve)
Diffusion of Na^+ in water	Spontaneous	$\Delta H = 0$

Thus, consideration of enthalpy (or internal energy) does not give us any information about the direction of a chemical reaction. In fact, in any isolated system (such as the universe) $\Delta H = 0$ from the 1st law, yet changes do occur in the universe.

The 1st law of thermodynamics can be thought of as an accounting tool, used to keep track of energy during a reaction.

CHANGE AND WORK

Change, such as a chemical reaction, is an orderly process.

Work (w) $\Rightarrow$ energy associated with an orderly process

Heat (q) $\Rightarrow$ energy associated with a disorderly process

Thus, when considering change we need to consider work energy not total energy.

For a change to occur, we need to either;

1. Put work energy into the system (drive change externally).

or;

2. Use some of the work energy already present in the system (spontaneous change).

If there is not enough available work energy, no change can occur.

2nd LAW OF THERMODYNAMICS

Spontaneous changes are those which, if carried out under the proper conditions, can be made to do work. If carried out reversibly they yield a maximum amount of work. In irreversible (spontaneous) processes the maximum work is never achieved.

ENTROPY

We need a measure of the available work energy in a system. We do this by determining the unavailable (non work) energy in a system. This is just the heat energy, q.

We define the ENTROPY, S, of a system as

$$dS = \frac{dq_{reversible}}{T} \quad \text{or} \quad \Delta S = \int_{initial}^{final} \frac{dq_{reversible}}{T}$$

S is a measure of the randomness of the system. $T\Delta S$ is a measure of the change in the heat energy of the system (energy unavailable to do any further work).

For any system;

Change in total energy = Change in work energy + Change in heat energy

ΔH - change in total energy (after allowing for expansion)

$T\Delta S$ - change in heat energy

Thus;

ΔH = Change in work energy + $T\Delta S$

or;

Change in work energy = ΔH - $T\Delta S$

If a change has occurred, then we must have used work energy to make the change happen and so the change in work energy must be -ve.

Isolated system :-

There is no external source of energy. Only internal work energy can be used.

$\Delta H = 0 \Rightarrow$ Change in work energy = $-T\Delta S$

The amount of work energy can never increase. Thus;

$$\Delta S \geq 0$$

If $\Delta S = 0$, there is no work energy being used and so the system does not change (equilibrium).
If $\Delta S > 0$, work energy is being used and so the system can change.

Closed systems :-

External energy is available.

$\Delta H \neq 0 \Rightarrow$ Change in work energy = $\Delta H - T\Delta S$

The amount of work energy can never increase during a change. Thus $\Delta S \geq \Delta H/T$.

GIBBS FREE ENERGY

Change in work energy = ΔH - $T\Delta S$

This can be rewritten as;

Change in work energy = $(H_{final} - TS_{final})$ - $(H_{initial} - TS_{initial})$

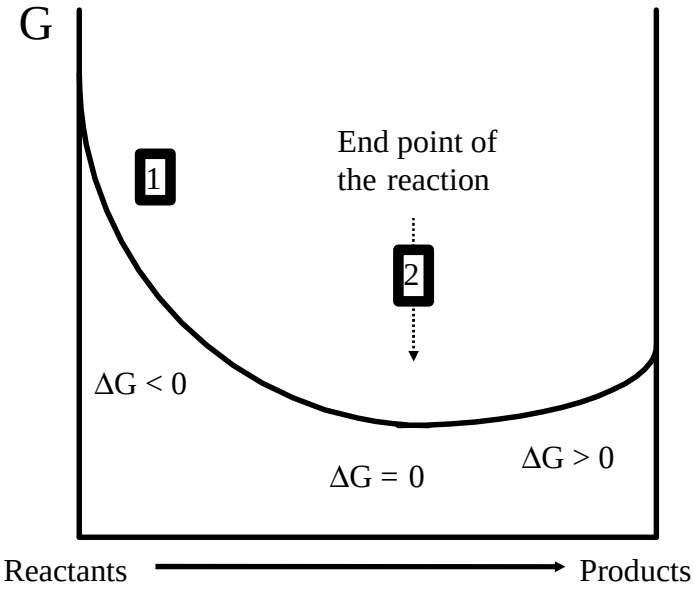
(H - TS) is an extensive state function and for convenience we call it the GIBBS FREE ENERGY, G.

Change in work energy = ΔG

At equilibrium $\Delta G = 0$. For an irreversible (spontaneous) reaction $\Delta G < 0$. This gives us a general criteria for whether a reaction will occur or not in a closed or isolated system.

POSITION OF EQUILIBRIUM

ΔG can be used to determine how far a reaction will go by letting the system change a small amount and then recalculating ΔG.



CONTROL OF REACTIONS

Kinetic control - 1

The products are not in equilibrium with the reactants. [This does not mean that an equilibrium cannot be established, only that is has not been established so far].

The amount of product depends on the amount of reactant, the speed of the reaction and how long it has been going.

The reaction cannot be made to go backwards by increasing the concentration of the products (until after equilibrium has been established).

Thermodynamic control - 2

The products are in equilibrium with the reactants.

The ratio of products to reactants depends on the relative energies of the two states (see later lectures).

The reaction can be made to go forwards or backwards by increasing the concentrations of the reactants or products respectively.

SINGLE COMPONENT SYSTEMS

A system consisting of one chemical can exist in three states, solid liquid and gas.

H - total energy in the system is related to the energy involved in the bonds between the molecules;

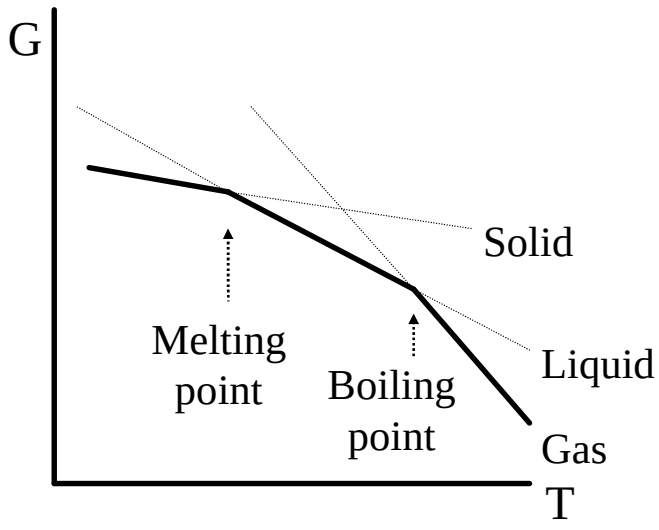
- Solid - H small and positive
- Liquid - H medium and positive
- Gas - H large and positive (lots of energy necessary to overcome bonds)

S - the randomness of the system depends on how free the molecules are to move around relative to each other;

- Solid - S small
- Liquid - S medium
- Gas - S large

PHASE CHANGES

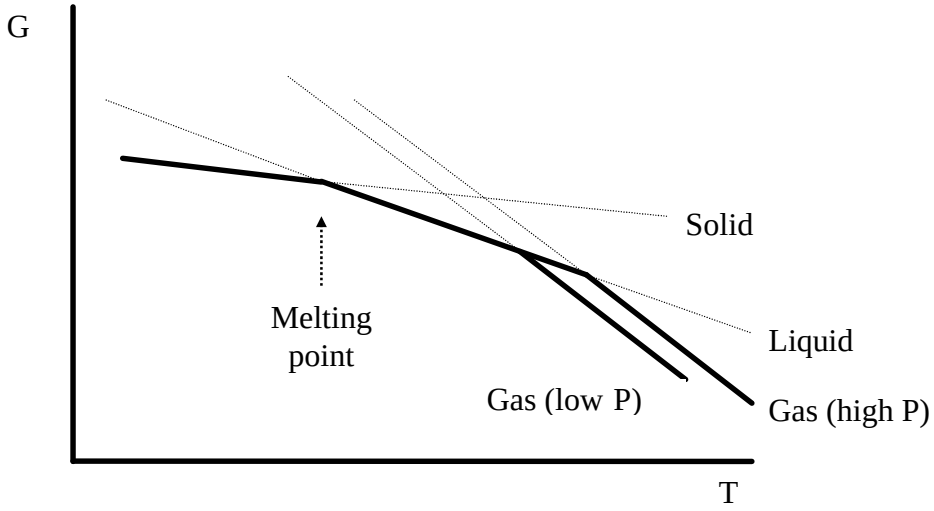
A plot of G versus temperature for any given phase will be a straight line with a slope of -S (G = H - TS). At any given temperature, the most stable phase will be the one with the lowest value of G.



EFFECTS OF PRESSURE ON PHASE EQUILIBRIA

Solid and liquid - virtually incompressible, thus pressure has no effect.

Gas - increasing the pressure will effect the properties of the molecules (they will collide more often). By compressing the gas, work is being done on the system and so G goes up. Note - it does not matter how you increase the pressure (e.g. compressing the system, adding another inert gas, etc.)



For an ideal gas -

$$G = G^\circ + RT \ln (P/P^\circ)^n$$

- Free energy of the compound
- Free energy of the compound in the standard state - depends on its chemical properties
- Variation in free energy of the compound with composition - depends on chemical environment

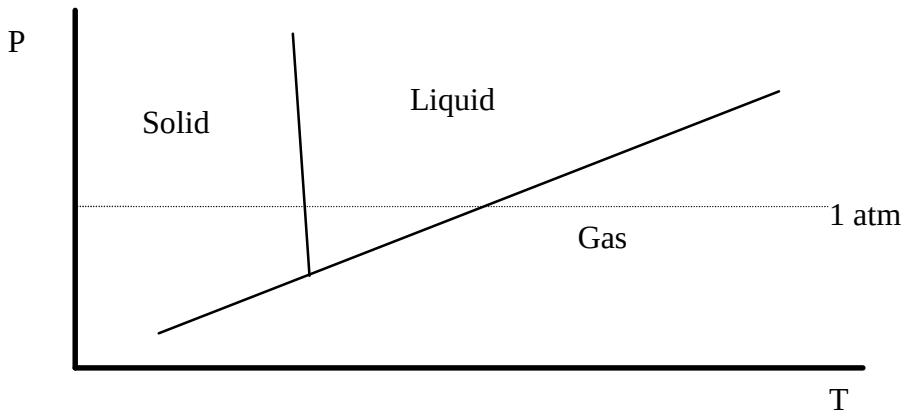
PHASE DIAGRAM FOR WATER

Plot of the most stable phase at a given temperature and pressure.

Solid-liquid phase change - independent of pressure (to a first approximation).

Liquid-gas phase change - occurs at a lower temperature as the pressure is reduced.

Below a given pressure, the liquid phase will not be stable and the solid will convert directly to the gas.



CONCENTRATION/DRYING OF PROTEINS

1. Increase the temperature so the liquid boils - the heat denatures the protein.

2. Reduce the pressure so that the liquid boils - this causes bubbling which denatures the protein.

3. Just leave to evaporate - at very low water content, the protein structure changes to compensate for the lack of solvent (denatures). This is also a problem with 1 and 2.

4. Freeze the sample to form a solid (usually at about 80 K) and then reduce the pressure so that the solid changes directly to a gas (called lyophilisation) - leaves the protein structure intact.

This technique is also used for;

- Drying/concentrating other biological samples (e.g. DNA)
- Drying foods for later rehydration (coffee, astronauts rations)
- Recovering paper after water damage

PROTEIN FOLDING / UNFOLDING

Folded protein :-

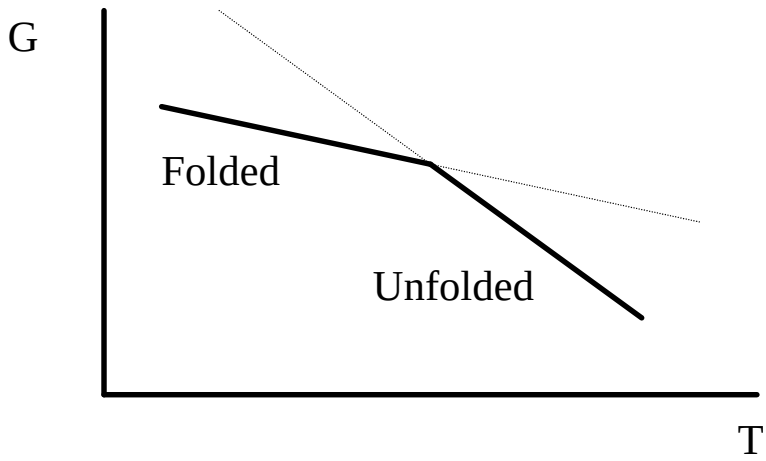
H - small and positive

S - small and positive

Unfolded protein :-

H- large and positive

S - large and positive



EFFECT OF PRESSURE ON G FOR A GAS
- derivation

$$G = H - TS \quad : \quad H = U + PV$$

Thus;

$$G = U + PV - TS$$

A very small change in G is given by;

$$\delta G = \delta U + P\delta V + V\delta P - T\delta S - S\delta T$$

For a closed system, from the 1st Law;

$$\delta U = \delta q - P\delta V$$

At equilibrium, from the 2nd Law;

$$\delta q = T\delta S \quad \Rightarrow \quad \delta U = T\delta S - P\delta V$$

Combining all of these gives;

$$\delta G = V\delta P - S\delta T$$

At constant temperature, $\delta T = 0$. Thus;

$$\delta G = V\delta P$$

For a perfect gas PV=nRT. Thus;

$$\delta G = nRT/P\ \delta P$$

Integrating this gives;

$$\int_{initial}^{final} dG = nRT \int_{initial}^{final} \frac{1}{P} dP$$

Thus;

$$G_{final} - G_{initial} = RT\ \mathrm{In}\left(\frac{P_{final}}{P_{initial}}\right)^n$$

Note that $\left(\frac{P_{final}}{P_{initial}}\right)$ is a dimensionless quantity, otherwise we could not take logs of it.

We normally take the initial state to be the standard state of the gas. Thus;

$$G_{initial} = G^\circ \quad : \quad P_{initial} = P^\circ = 1\ \text{atm}$$

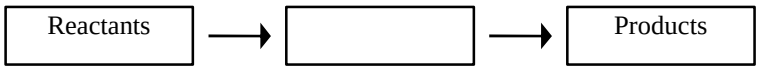
Thus;

$$G = G^o + RT\ \mathrm{In}\left(\frac{P}{P^o}\right)^n$$

CHEMICAL REACTIONS

Thermodynamics is only interested in comparing the state of a system before and after a change has occurred (and is not interested in the mechanism of that change).

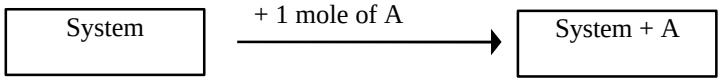
A chemical reaction, in thermodynamic terms, simply consists of taking away reactants and adding products.



$\Delta G = \Delta G$ (removing reactants) + ΔG (adding products)

CHEMICAL POTENTIAL

The chemical potential of a species is defined as the change in free energy (G) of a system when 1 mole of the species is added to the system at constant temperature and pressure†. It is an intensive state function.



ΔG = Chemical potential of A (μ_A)

If instead of adding 1 mole of A we add δn_A moles of A, then

$$\Delta G = \mu_A \,.\, \delta n_A$$

For any chemical reaction, ΔG (at constant temperature and pressure) is simply given by adding up the changes in each component.

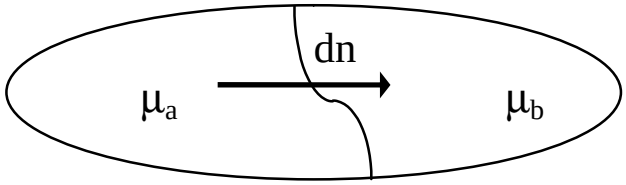
$$\Delta G = \Sigma_i \, \mu_i \, \delta n_i$$

† Note - more formally, μ is the rate of change of G with composition.

$$m_i = \left(\frac{dG}{dn_i} \right)_{T,P,n_j}$$

EQUILIBRIUM AND CHEMICAL POTENTIAL

Consider a system consisting of a component distributed between two phases, a and b. The chemical potentials in the two phases are μ_a and μ_b. At constant temperature and pressure, if we transfer δn moles from a to b then;



$\Delta G_a = - \mu_a . \delta n$ -- (negative because matter is being lost)

$\Delta G_b = + \mu_b . \delta n$ -- (positive because matter is being gained)

Overall;

$$\Delta G = \Delta G_b + \Delta G_a = \mu_b.\delta n - \mu_a.\delta n$$

$$\Delta G = (\mu_b - \mu_a).\delta n$$

At equilibrium, $\Delta G = 0$. Thus;

$$\mu_b = \mu_a.$$

Equalising the chemical potentials is the driving force for any reaction.

VARIATION IN μ WITH COMPOSITION

For a pure substance

$$\mu = G \quad \text{and} \quad \mu^o = G^o$$

by definition. Thus, for a pure gas (by analogy with the equation for G);

$$\mu = \mu^\circ + RT \ln (P/P^\circ)$$

Unlike G, we can also use μ to describe a single component in a mixture. For one species (i) in a mixture of perfect gases;

$$\mu_i = \mu_i^\circ + RT \ln (P_i/P^\circ)$$

where P_i is the partial pressure of i.

We can derive a very similar equation for liquids and solutions;

$$\mu_x = \mu_x^\circ + RT \ln ([x]/[x]^\circ)$$

where [x] is the concentration of x in the liquid and $[x]^\circ$ is its concentration in its standard state.

For solids, G does not change and so neither does μ ($\mu_i = \mu_i^\circ$).

FREE ENERGY OF A REACTION

ΔG for a chemical reaction at constant pressure and temperature is given by;

$$\Delta G = \Sigma_i \mu_i \delta n_i$$

For each component;

$$\mu_x = \mu_x^\circ + RT \ln ([x]/[x]^\circ)$$

Thus;

$$\Delta G = \sum_x \mu_x^o \delta n_x + \sum_x RT \delta n_x \ln\left(\frac{[x]}{[x]^o}\right)$$

$$= \sum_x \mu_x^o \delta n_x + RT \ln\left(\prod_x \left(\frac{[x]}{[x]^o}\right)^{\delta n_x}\right)$$

δn_x is negative for reactants and positive for products.

$\Sigma_x \mu_x^\circ \delta n_x$ is the standard free energy change for a mole of reactants giving a mole of products, ΔG°.

δn_x for a complete reaction is the stoichiometry coefficient (m_x) for that component.

The [x]° terms are often left out because they usually equal 1.0 (although this can lead to confusion).

Thus;

$$\Delta G = \Delta G^{o} + RT \ln \left(\frac{\prod_{products} \left(\frac{[x]}{[x]^{o}} \right)^{m_x}}{\prod_{reactants} \left(\frac{[x]}{[x]^{o}} \right)^{m_x}} \right)$$

EQUILIBRIUM CONSTANTS

At equilibrium, the ratio of products to reactants is called the equilibrium constant, K.

$$K = \left(\frac{\prod_{products} \left(\frac{[x]}{[x]^{o}} \right)_{eq}^{m_x}}{\prod_{reactants} \left(\frac{[x]}{[x]^{o}} \right)_{eq}^{m_x}} \right)$$

K is a dimensionless quantity (it has no units), but [x] must have the same units as [x]°.

At equilibrium $\Delta G = 0$, by definition. Thus;

$$\Delta G^{\circ} = -RT \ln (K)$$

or in the biological standard state;

$$\Delta G^{\circ\prime} = -RT \ln (K')$$

Note - ΔG° and $\Delta G^{\circ\prime}$ (and thus K and K′) are only different if H^+ is involved in the reaction because x° is different for H^+.

HYDROLYSIS OF ATP

Consider the hydrolysis of ATP to ADP and inorganic phosphate;

$$ATP\ (aq) + H_2O \leftrightarrow ADP\ (aq) + P_i\ (aq) + H^+\ (aq)$$

Measuring concentrations of an equilibrium mixture of ATP, ADP and P_i at pH = 7 and T = 310K gives K' = 1.3×10^5 (M). Thus;

$$K' = \frac{[ADP]_{eq}[P_i]_{eq}[H^+]_{eq}}{[ATP]_{eq}[H_2O]_{eq}} = \frac{[ADP]_{eq}[P_i]_{eq}}{[ATP]_{eq}} = \exp\left(\frac{-\Delta G^{o'}}{RT} \right)$$

and so $\Delta G^{o'} = -30.5$ kJ mol^{-1}.

Note: $[H^+] = [H_2O] = 1.0$ because they are in their standard states (pH = 7.0 for the former and approximately a pure liquid for the latter). If we were considering K rather than K′, then $[H^+]$ would equal 10^{-7} at pH=7.0.

Hydrolysis of ATP to ADP in cells :-

The concentrations of ATP, ADP and P_i in a cell are found to be;

$[ATP] = 1 \times 10^{-2}$ mol.dm^{-3}
$[ADP] = 3 \times 10^{-3}$ mol.dm^{-3}
$[P_i] = 1 \times 10^{-3}$ mol.dm^{-3}

If the system were at equilibrium;

$$[ATP]_{eq} = \frac{[ADP]_{eq}[P_i]_{eq}}{K'} = 2.3 \times 10^{-11} \text{ mol dm}^{-3}$$

Thus, the cell is not at equilibrium. If it were at equilibrium, no work could be obtained from the hydrolysis of ATP ($\Delta G = 0$).

ΔG in the cell is given by;

$$\Delta G = \Delta G^{o'} + RT \ln\left(\frac{[ADP][P_i]}{[ATP]}\right) = -51.4 \text{ kJ mol}^{-1}$$

ΔG is large and negative, thus considerable work can be done in the cell by hydrolysing ATP.

MASS ACTION RATIO (MAR)

The mass action ratio is defined as;

$$MAR = \frac{\prod_{products}[x]^m}{\prod_{reactants}[x]^m}$$

Thus;

$$\Delta G = \Delta G° + RT \ln (MAR)$$

If the system is at equilibrium,

$$MAR = K$$

If the system is not at equilibrium then the difference between the MAR and K gives a measure of how far from equilibrium the system is.

$$\Delta G = RT \ln(MAR/K)$$

As a rough rule of thumb, if MAR is more than 2 orders of magnitude (100 times) smaller than K in a biological system, then that reaction is likely to be kinetically controlled rather than thermodynamically controlled.

HYDROLYSIS OF ATP -- again

$$ATP\ (aq) + H_2O \leftrightarrow ADP\ (aq) + P_i\ (aq) + H^+\ (aq)$$

For this reaction;

$$K' = \frac{[ADP]_{eq}[P_i]_{eq}[H^+]_{eq}}{[ATP]_{eq}[H_2O]_{eq}} = 1.3 \times 10^5$$

For the observed concentrations of ATP, ADP and Pi in a cell;

$[ATP] = 1 \times 10^{-2}$ mol.dm^{-3}
$[ADP] = 3 \times 10^{-3}$ mol.dm^{-3}
$[P_i] = 1 \times 10^{-3}$ mol.dm^{-3}

Thus;

$$MAR = \frac{[ADP]\,[P_i]\,[H^+]}{[ATP]\,[H_2O]} = 3 \times 10^{-4}$$

High-energy phosphate bonds;

Because hydrolysis of ATP involves breaking a phosphate bond and the equilibrium lies along way to the right, this is called a high-energy phosphate bond, and is often interpreted as the phosphate bond being weak.

It requires a great deal of energy to break a bond (for O-P ≈ 540 kJ mol^{-1}). If energy was given out when a bond is broken, then the bond would never be formed in the first place.

Thermodynamics (in this case K and ΔG) does not give us any information about a small part of the system, only about the difference between the state of the whole system before and after the reaction.

$$\Delta G = G_{final} - G_{initial} = \Delta H - T\Delta S$$

In this case;

ΔH - depends on the stability of the reactants and the products, one component of which is the energy of the bonds that need to be broken or formed (including with the solvent).

ΔS - depends on the number of reactant and product molecules and on the ordering effect they have on the solvent.

MEASUREMENT OF THERMODYNAMIC QUANTITIES

K	Measure concentrations at equilibrium.
ΔG^o	Calculate from $\Delta G^o = -RT \ln(K)$.
ΔG	Measure concentrations and calculate from $\Delta G = \Delta G^o + RT \ln(MAR)$.
ΔH^o	Measure temperature dependence of K.
ΔH	Measure directly by calorimetry.
ΔS^o	Calculate from $\Delta G^o = \Delta H^o - T\Delta S^o$
ΔS	Calculate from $\Delta G = \Delta H - T\Delta S$

ΔG^o, ΔH^o, ΔS^o can also be calculated via Hess's Law from standard values.

VARIATION OF EQUILIBRIUM CONSTANT WITH TEMPERATURE

As $\Delta G^o = - RT \ln(K)$, then;

$$\ln K = -\frac{\Delta G^o}{RT} = -\frac{\Delta H^o}{RT} + \frac{\Delta S^o}{R}$$

Assuming that ΔH^o and ΔS^o are independent of temperature and differentiating with respect to T gives;

$$\frac{d \ln K}{dT} = \frac{\Delta H^o}{RT^2}$$

This equation is called the Van't Hoff Isochore. Reintegrating this equation gives;

$$\ln\left(\frac{K_2}{K_1}\right) = -\frac{\Delta H^o}{R}\left(\frac{1}{T_2} - \frac{1}{T_1}\right)$$

Thus, a plot of ln(K) vs. 1/T should give a straight line of slope $-\Delta H^o/R$.

MULTIPLE STEP REACTIONS

Many reactions involve several discrete steps.

Reactants $\leftrightarrow$ Intermediates $\leftrightarrow$ Products

In this case, we can apply our standard thermodynamic analysis to each step in turn.

Reactants $\leftrightarrow$ Intermediates ....(1)

$$\Delta G(1) = \Delta G(1)^o + RT \ln\left(\frac{\prod_{intermediates}[intermediates]^n}{\prod_{reactants}[reactants]^n}\right)$$

and

Intermediates $\leftrightarrow$ Products ....(2)

$$\Delta G(2) = \Delta G(2)^o + RT \ln\left(\frac{\prod_{products}[products]^n}{\prod_{intermediates}[intermediates]^n}\right)$$

However, since G is a state function (it only depends on how the system is at the start and at the end), we must also be able to apply our standard equations to the whole reaction regardless of what the intermediates are.

Reactants $\leftrightarrow$ *{Intermediates}* $\leftrightarrow$ Products ....(3)

$$\Delta G(3) = \Delta G(3)^{o} + RT \ln \left(\frac{\prod_{products} [products]^{n}}{\prod_{reactants} [reactants]^{n}} \right)$$

From this it also follows that;

$\Delta G(3) = \Delta G(1) + \Delta G(2)$ and $\Delta G(3)° = \Delta G(1)° + \Delta G(2)°$

COUPLED REACTIONS

Sequential reactions :-

$$A \leftrightarrow B \leftrightarrow C \quad : \quad \Delta G\,(AB) > 0, \Delta G(BC) << 0$$

$$\Delta G\,(AC) = \Delta G\,(AB) + \Delta G\,(BC) < 0$$

Thus, A will still be converted into C although the conversion from A to B is unfavourable.

Parallel reactions :-

$$A \leftrightarrow B \quad : \quad \Delta G\,(AB) > 0$$
$$C \leftrightarrow D \quad : \quad \Delta G\,(CD) << 0$$

The first reaction would normally not proceed, the second would proceed. If the two reactions are coupled, then;

$$A + C \leftrightarrow B + D \quad : \quad \Delta G = \Delta G\,(AB) + \Delta G\,(CD) < 0$$

and the reaction will proceed converting A into B.

GLUTAMINE SYNTHESIS

Glutamine, used both as an amino-acid and a non-toxic source of ammonia for other biochemical pathways, is produced in cells from glutamate.

$$Glutamate(aq) + NH_3(aq) \leftrightarrow Glutamine(aq) + H_2O \qquad \Delta G°' = + 14.3 \text{ kJ mol}^{-1}$$

This reaction would not normally proceed. However, the free energy necessary to drive the reaction forward 14.3 kJ mol^{-1} is less than the free energy given out by hydrolysis of ATP.

$$ATP(aq) + H_2O \leftrightarrow ADP(aq) + P_i(aq) \qquad \Delta G°' = - 30.5 \text{ kJ mol}^{-1}$$

Thus, the coupled reaction;

$$ATP(aq) + Glutamate(aq) + NH_3(aq) \leftrightarrow ADP(aq) + P_i(aq) + Glutamine(aq)$$

$$\Delta G°' = + 14.3 - 30.5 = - 16.2 \text{ kJ mol}^{-1}$$

will proceed spontaneously. These two reactions are coupled together by the enzyme glutamine synthetase

THE TRIOSE PHOSPHATE ISOMERASE REACTION

The reaction

$$\underset{\text{1,6-bisphosphate}}{\text{fructose}} \rightleftharpoons \underset{\text{phosphate}}{\text{dihydroxyacetone}} + \underset{\text{3-phosphate}}{\text{glyceraldehyde}}$$

has an equilibrium constant of 1.5×10^{-3} and is catalysed by the enzyme aldolase. Thus, it appears that this equilibrium lies to the left.

The next step in the glycolytic pathway uses G-3-P and so dha-P needs to be converted to G-3-P. The reaction

$$\underset{\text{phosphate}}{\text{dihydroxyacetone}} \rightleftharpoons \underset{\text{3-phosphate}}{\text{glyceraldehyde}}$$

has an equilibrium constant of 0.04 and is catalysed by the enzyme triose phosphate isomerase. This equilibrium also appears to lie to the left.

Aldolase is added to a 4 mM solution of fructose 1,6-bisphosphate. At equilibrium, assume x mM of G-3-P has been produced, so the equilibrium concentrations are:

$$[\text{F-1,6-P}] = 4\text{-x mM} \quad : \quad [\text{G-3-P}] = [\text{dha-P}] = \text{x mM}$$

Thus

$$K = \frac{[\text{dha-P}][\text{G-3-P}]}{[\text{F-1,6-P}]} = \frac{(x\times10^{-3})^2}{(4-x)\times10^{-3}} = 1.5\times10^{-3}$$

This gives

$$[\text{F-1,6-P}] = 2.23 \text{ mM} \quad : \quad [\text{G-3-P}] = [\text{dha-P}] = 1.77 \text{ mM}$$

Even though the equilibrium constant is small (1.5×10^{-3}), at these concentrations the reaction proceeds to approx. 50%.

Aldolase and triose phosphate isomerase are added to a 4 mM solution of fructose 1,6-bisphosphate. At equilibrium, assume x mM of F-1,6-P has been used and y mM of G-3-P has been converted to dha-P, so the equilibrium concentrations are:

$$[\text{F-1,6-P}] = 4\text{-x mM} \quad : \quad [\text{G-3-P}] = \text{x–y mM} \quad : \quad [\text{dha-P}] = \text{x+Y mM}$$

Thus;

$$\frac{(x-y)\times10^{-3}\times(x+y)\times10^{-3}}{(4-x)\times10^{-3}} = 1.5\times10^{-3} \quad \text{and} \quad \frac{(x-y)\times10^{-3}}{(x+y)\times10^{-3}} = 0.04$$

This gives

$$[\text{F-1,6-P}] = 0.93 \text{ mM} \quad : \quad [\text{G-3-P}] = 0.24 \text{ mM} \quad : \quad [\text{dha-P}] = 5.90 \text{ mM}$$

Addition of triose phosphate isomerase to the products of the aldolase reaction lowers the equilibrium concentration of G-3-P by nearly a factor of 10.

At first sight this is unfavourable, as G-3-P is the reactant for the next step of the glycolytic pathway. However, this subsequent reaction will reduce the concentration of G-3-P (i.e. is sequentially coupled to the aldolase and triose phosphate isomerase reactions), which will result in more dha-P being converted to G-3-P. In the context of the whole pathway triose phosphate isomerase increases the thermodynamic efficiency by enabling dha-P to be used.

BIOSYNTHETIC PATHWAYS

A biosynthetic pathway consists of a series of sequentially coupled reactions. This enables the free energy from one reaction to be used by another reaction.

- Reactions that are only slightly unfavourable thermodynamically are driven by sequential coupling in the pathway.

- Reactions that have very low equilibrium constants are coupled directly (in parallel) to energy producing reactions, such as ATP hydrolysis.

One of the evolutionary selection pressures is the availability of energy, and so pathways need to be reasonably efficient from a thermodynamic point of view.

ΔG°' FOR GLYCOLYSIS

The net reaction for glycolysis is

$$\text{glucose} + 2\ NAD^+ + 2\ ADP + 2\ P_i \rightleftharpoons 2\ \text{pyruvate} + 2\ NADH + 2\ ATP + 2\ H^+ + 2\ H_2O$$

The free energies of formation of the reactants and products are

	ΔG°'(formation) (kJ mol^{-1})		ΔG°'(formation) (kJ mol^{-1})
ADP	-1409.00	NAD^+	1059.11
ATP	-2276.77	NADH	1120.09
Glucose	-426.71	P_i	-1059.49
H^+	0.00	Pyruvate	-350.78
H_2O	-155.66		

Thus

$$\Delta G^{\circ\prime} = \Delta G^{\circ\prime}_f(\text{products}) - \Delta G^{\circ\prime}_f(\text{reactants}) = -80.77\ \text{kJ mol}^{-1}$$

ΔG FOR GLYCOLYSIS

The metabolite concentrations measured in erythrocytes are as follows:

	Concentration (mM)		Concentration (mM)
ADP	0.14	P_i	1.00
ATP	1.85	Pyruvate	0.05
Glucose	5.00		

Assuming that the NAD^+/NADH ratio is 1 and the cytoplasmic pH is 7,

$$\Delta G = \Delta G^{\circ'} + RT\ \ln \frac{[\text{pyruvate}]^2[ATP]^2[NADH]^2}{[\text{glucose}][ADP]^2[Pi]^2[NAD^+]^2} = -69.6\ \text{kJ mol}^{-1}$$

This corresponds to the free energy released (wasted) during the glycolytic pathway (i.e. about one ATPs worth over 10 coupled reactions). Infact, this energy is not all wasted because it is necessary to make the pathway (i) irreversible (otherwise a small decrease in glucose concentration would lead to glucose synthesis) and (ii) proceed at a reasonable rate.

THE GLYCOLYTIC PATHWAY

Theory of Flux Generating Steps –

Flux through (kinetics of) a biosynthetic pathway is a measure of the overall rate for conversion of the initial reactant into the final product. This is governed by reactions that are not in equilibrium (under kinetic control). These have two functions;

- They make the pathway irreversible (it cannot go backwards).
- They indirectly control the reactions that are in equilibrium.

Reactions that are at equilibrium play no role in controlling the flux.

Step	K	MAR		MAR/K
		Brain	Heart	
Hexokinase	$3.09\text{-}5.50 \times 10^{3}$	0.04	0.08	10^{-5}
PGI	0.36-0.47	0.22	0.24	1
PFK	$0.09\text{-}1.20 \times 10^{2}$	0.13	0.03	10^{-3}
Aldolase	$6.08\text{-}13.0 \times 10^{-5}$	2.4×10^{-6}	9×10^{-6}	10^{-1}
TIM	$3.06\text{-}4.50 \times 10^{-2}$	-	0.24	10
GAPDH	$0.02\text{-}1.50 \times 10^{3}$	5.3	9.0	10^{-2}
PGM	0.01-0.02	0.2	0.12	1
Enolase	2.08-4.60	3.6	1.4	1
Pyruvate kinase	$0.20\text{-}2.00 \times 10^{4}$	5.4	40.0	10^{-3}

The theory of flux generating steps is flawed because it analyses reactions independently. As we have seen, at a thermodynamic level (and a kinetic level) all reactions in a pathway are coupled and the system needs to be analysed as a whole. This approach is called Metabolic Control Analysis.