

Development of Complex Curricula for Molecular Bionics and Infobionics Programs within a consortial* framework**

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**Molekuláris bionika és Infobionika Szakok tananyagának komplex fejlesztése konzorciumi keretben

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INTRODUCTION TO BIOPHYSICS

(Bevezetés a biofizikába)

THERMODYNAMICS OF SOLUTIONS

(Oldatok termodinamikája)

GYÖRFFY DÁNIEL, ZÁVODSZKY PÉTER

Introduction

- Reactions in solutions are described by somewhat different laws than reactions of gases because molecules of solvent have a significant effect on the kinetics of reactions
- Although the number of collisions is the same in solutions as in gas phase, the rate of reactions can be very different
- Depending on the ratio of the rate of transformation following a collision to the rate of diffusion we can deviate two types of solution phase reactions

- For the components of a solution, partial molar quantities can be defined which show how the given quantity changes when an infinitesimal amount of a component is added to the solution
- Because of its importance, the partial molar free energy is called chemical potential
- Chemical potential tends to balance by flowing material

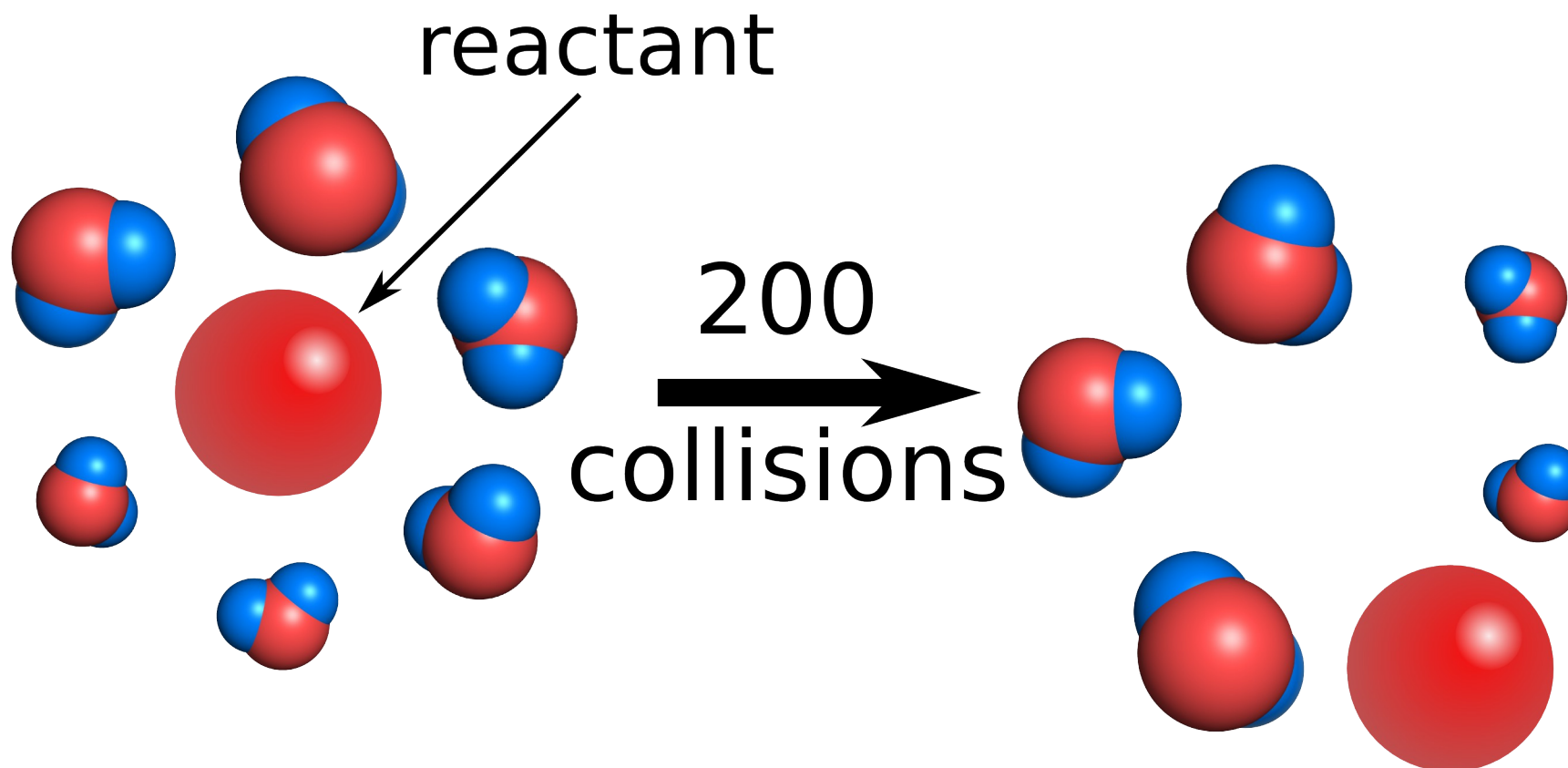
- In solutions, there is a relationship between the mole fraction of a component and its chemical potential
- For ideal (sufficiently diluted) solutions, this relation is linear
- Raoult's law states a proportionality between the mole fraction and the vapour pressure of a component of a solution
- For some diluted but not ideal solutions, this relationship is described by Henry's rather than Raoult's law

Reactions in solutions

- In a gas, significant interactions occur only between particles taking part in the chemical reaction
- In solutions, molecules of solvent also affect the kinetics of reaction so we have to take into account collisions of the reactant molecules with the solvent molecules

- The solvent molecules form a 'cage' around the reactant molecules
- For small solvent molecules such as water molecules, the reactant molecule collides about 200 times with the solvent cage before it diffuses a distance corresponding to its diameter

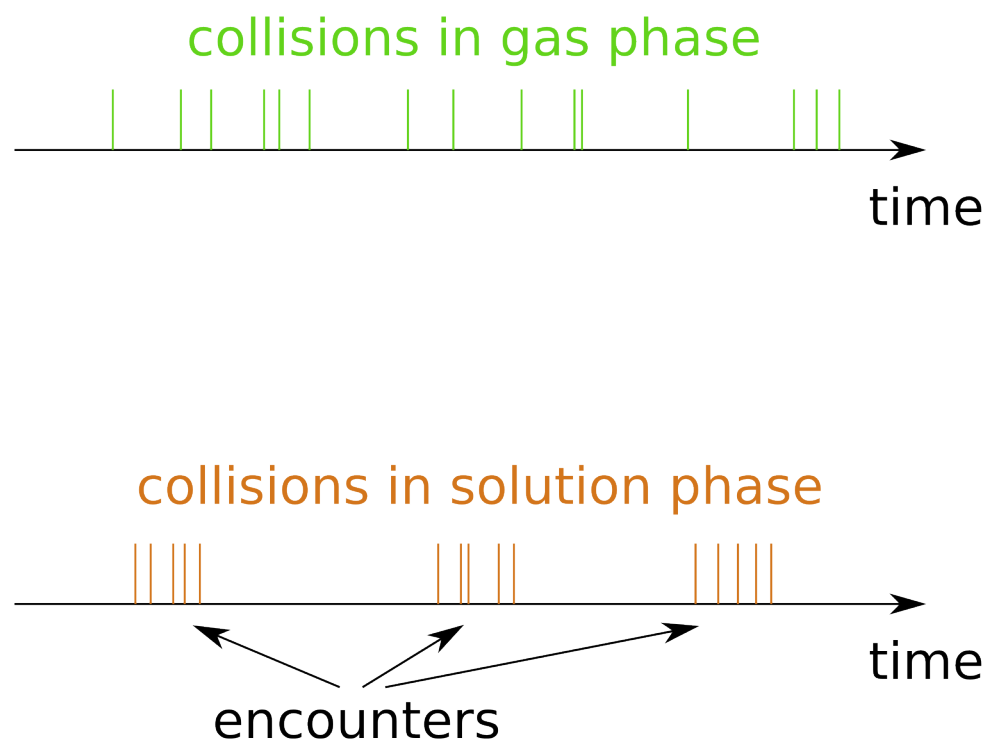
Reactant in a solvent milieu



- Eventually, two reactant molecules, A and B, should diffuse together and occupy the same solvent cage
- Then A and B will collide about 200 times in this one 'encounter' before they move away from each other
- This is in contrast to the gas phase reaction where A and B will collide only once before moving apart

- Now, let us compare the *collisions* in the gas and solution phases over time
- Each slash in the following figure represents a collision between A and B
- In gas phase, randomly uniform collisions occur while in solution phase collisions take place as 'packets' which are called '*encounters*'

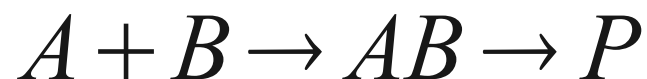
Comparison of the collision distributions of gas and solution phase reactions



- Over time, the total number of collisions for the gas and solution phase reactions are about the same
- It does however change the pattern of distribution of collisions

- Naturally, we are interested in how the presence of solvent affects the reaction rate
- For an improbable reaction – where the activation energy is large – which occurs only once in about 10^6 collisions, there is no difference, i.e. the reaction goes at the same rate in the solution phase as in the gas phase
- For a reaction with low activation energy, which occurs about once in every 10 collisions, the reaction in the solution phase will occur every time the molecules encounter each other

- Formally, let us consider a reaction between the reactants A and B



- The velocity of formation of an encounter complex, which can be assumed to be a first-order reaction with respect to both A and B

$$v = k_1 [A][B]$$

- Having formed the complex, the reactants can move away from each other or the reaction can occur



or



where P is the product of the reaction

- The concentration change rate of the encounter complex is

$$\frac{d[AB]}{dt} = k_1[A][B] - k_{-1}[AB] - k_2[AB]$$

- Using the steady-state approximation, the concentration of the encounter complex is obtained as

$$[AB] = \frac{k_1[A][B]}{k_{-1} + k_2}$$

- Thus the rate law for the formation of the product is

$$\frac{d[P]}{dt} \approx k_2 [AB] \approx \frac{k_2 k_1}{k_2 + k_{-1}}$$

- The case when $k_{-1} \ll k_2$, i.e. the rate of formation of the product is far larger than the disintegration of the encounter complex, corresponds to a low activation energy
- These reactions are called *diffusion controlled reactions*

- In contrast, the case when $k_2 \gg k_{-1}$, i.e. the rate of formation of the product is far smaller than the disintegration of the encounter complex, corresponds to a high activation energy
- These reactions are called *energy* or *activation controlled reactions*

Diffusion controlled reactions

- In every packet of collisions, which may contain about 100-200 collisions, there will certainly be one with sufficient energy and correct orientation
- For example, in an enzymatic reaction, the enzyme and the substrate will find each other and collide many times while rotating with respect to each other until they have the correct orientation and the reaction occurs
- This type of reaction occurs very fast

- Enzymatic reactions are limited only by how fast the reactants can diffuse towards each other, and collide
- To formalize the velocity of diffusion controlled reactions, let us consider a spherically symmetric system where the centre is occupied by a particle A and this particle is surrounded by B particles according to some distribution

- Let $[B](r)$ denote the concentration of particle B at the distance r
- Since we assume that every collision leads to a reaction, the concentration of B at r_{AB} is

$$[B](r_{AB})=0$$

where r_{AB} is the sum of the radii of A and B

- At infinite distance, the concentration of B approaches the value of bulk concentration of B

$$\lim_{r \rightarrow \infty} [B](r) = [B]$$

- Since the concentration of B continuously decreases as we approach A, there is a flux of B towards A

- This flux can be described by the *Fick's first law*:

$$\mathbf{J} = 4\pi r^2 D_{AB} \frac{d[B](r)}{dr}$$

where $\mathbf{J}$ is the flux, i.e. the amount of substances crossing a spherical unit area centred around A in unit time, $D_{AB} = D_A + D_B$ is the relative diffusion coefficient of A and B, r is the distance between A and B, and $d[B](r)/dr$ is the concentration gradient of B

Adolf Fick (1829-1901)



- To get the concentration of B as a function of r , we need to integrate the equation above from r to ∞

$$\int_{[B](r)}^{[B](\infty)} d[B](r) = \int_r^{\infty} \frac{\mathbf{J}}{4\pi r^2 D_{AB}} dr$$

- Thus

$$[B](r) = [B] - \frac{\mathbf{J}}{4\pi r D_{AB}}$$

- Since

$$[B](r_{AB})=0$$

the flux is

$$\mathbf{J} = 4\pi r_{AB} D_{AB} [B]$$

- Substituting the expression obtained for the flux into the equation for $[B](r)$ we get

$$[B](r) = [B] \left(1 - \frac{r_{AB}}{r} \right)$$

which is the distribution of concentration of B as a function of the distance from A

- Since the particle B which crosses the spherical surface with radius r_{AB} immediately reacts, we can calculate the velocity of the reaction based on the flux at r_{AB}
- The total velocity of the reaction is

$$v = \mathbf{J} [A]$$

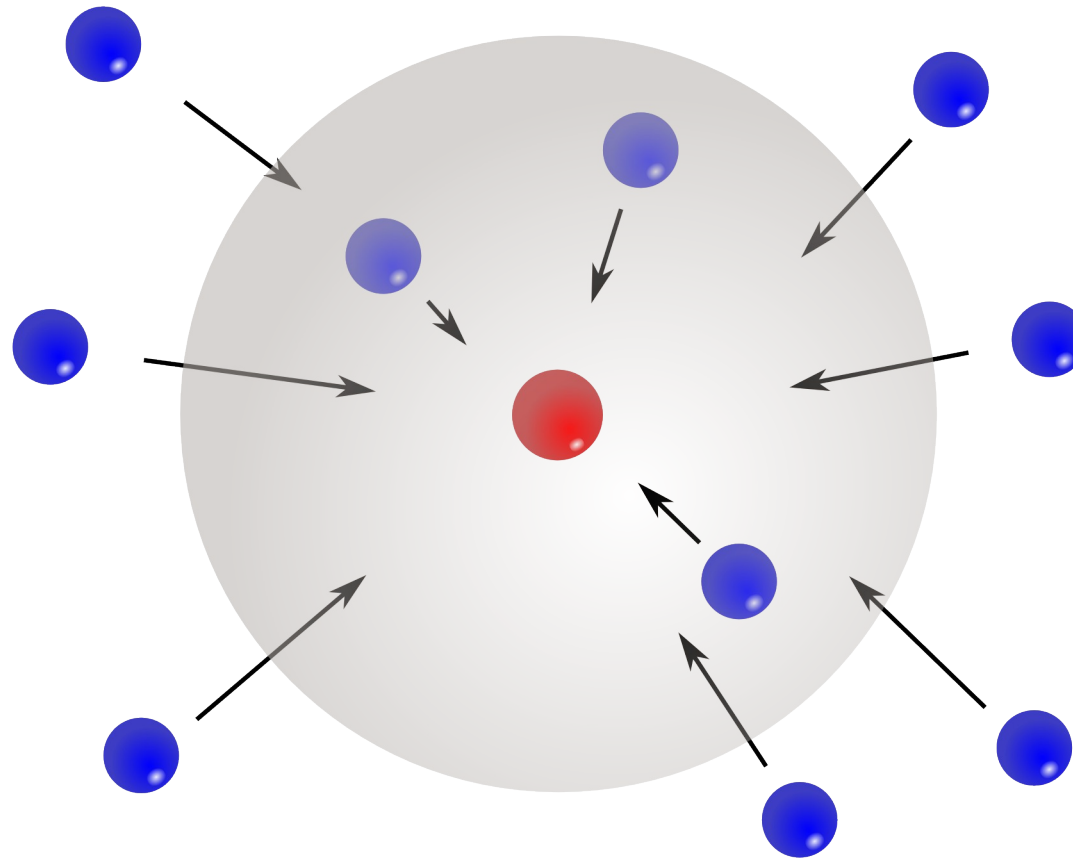
- And since

$$v = k [A][B]$$

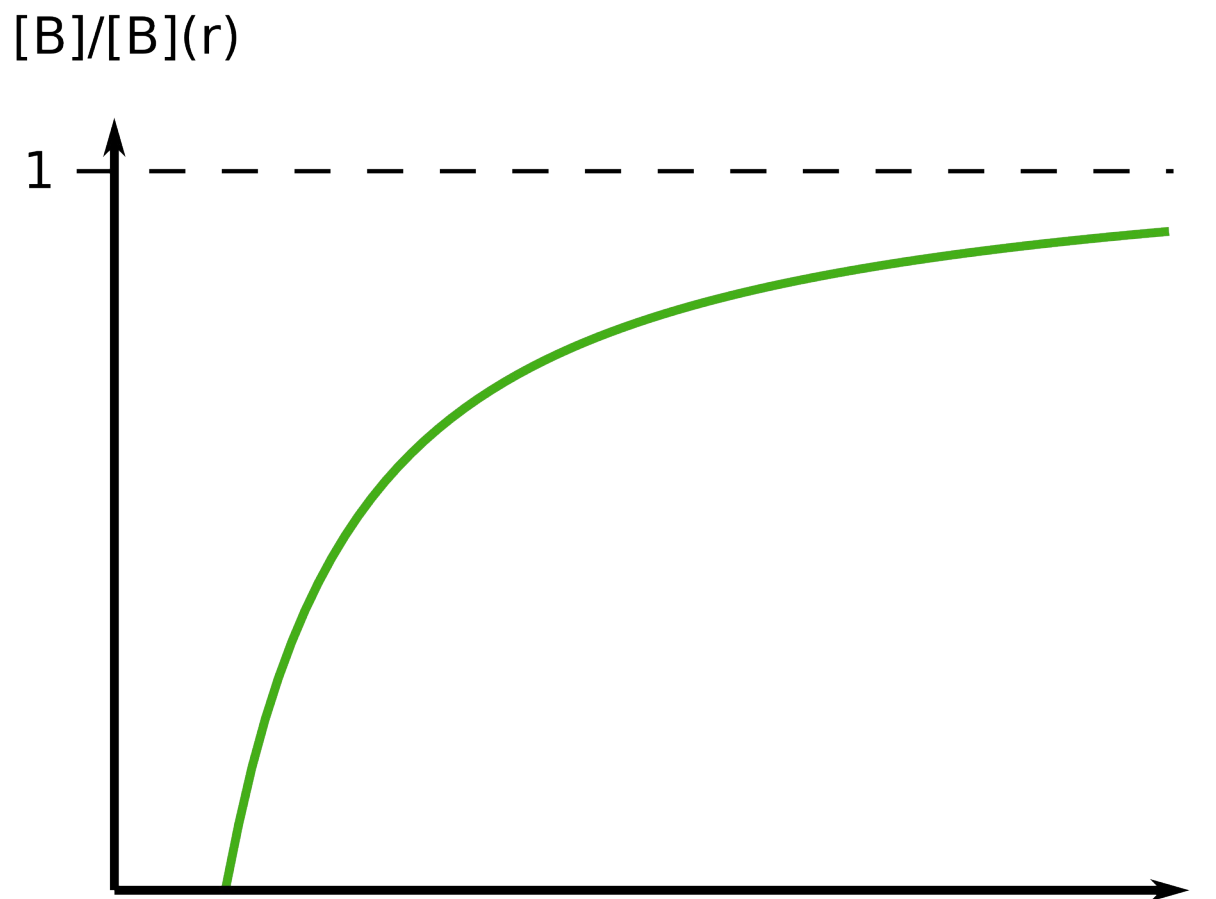
the rate constant is

$$k = \frac{v}{[A][B]} = \frac{J}{[B]} = 4\pi r_{AB} D_{AB}$$

Diffusion of B particles towards an A particle



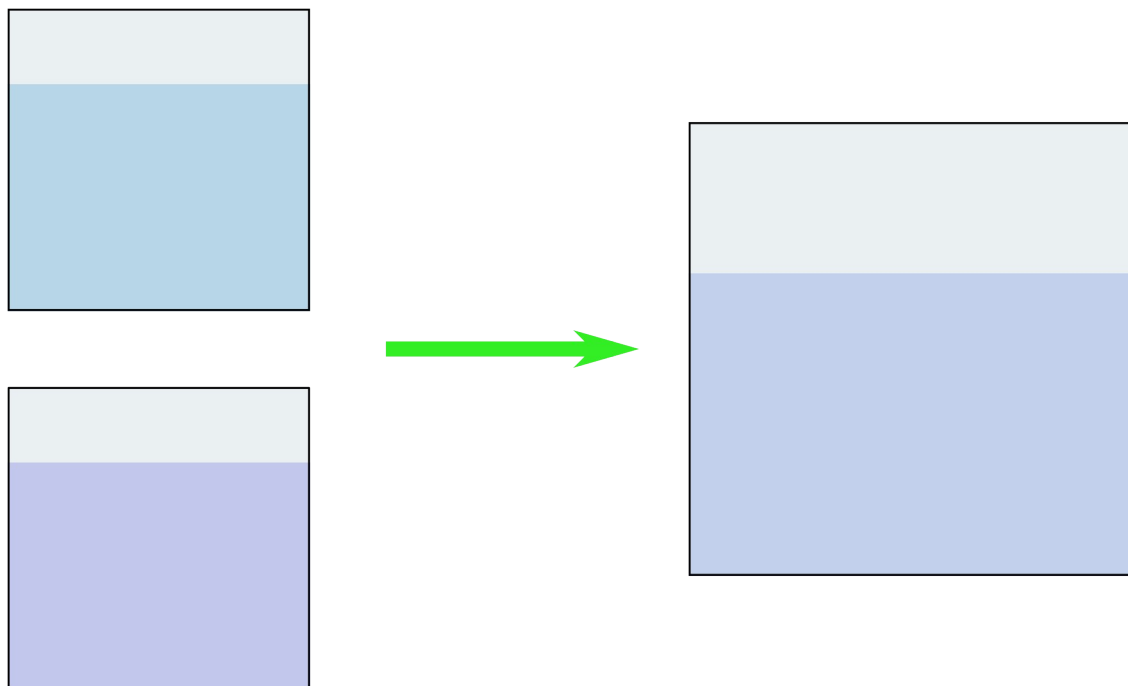
The concentration distribution of B particles around an A particle



Chemical potential

- Let us consider two containers containing solutions with different concentrations
- If they are mixed, we observe that the concentration becomes uniform
- We know that the equilibrium of a system where the temperature, the pressure and the amounts of the components are constant is characterized by the minimum of the Gibbs free energy

Mixing of solutions



- Based on these observations and knowledge it is obvious that the Gibbs free energy of a solution is a function of the amounts of components
- We know that the free energy is

$$G = U - TS$$

- According to the first law of thermodynamics the differential of internal energy is

$$dU = \delta q + \delta w$$

- The work in our simple case contains a term related to the volume and a term related to the amount of material of components

$$\delta w = \sum_i \mu_i dn_i - p dV$$

- Substituting the term describing the change of internal energy into the equation describing the change of Gibbs free energy, we get

$$dG = -SdT - pdV + \sum_i \mu_i dn_i$$

- If we consider dG as a total derivative, the expression above has the following form

$$dG = \left(\frac{\partial G}{\partial T} \right)_{p, n_j} dT + \left(\frac{\partial G}{\partial V} \right)_{T, n_j} dV + \sum_i \left(\frac{\partial G}{\partial n_i} \right)_{p, T, n_j \neq n_i} dn_i$$

- It is obvious based on the equations above that

$$\mu_i = \left(\frac{\partial G}{\partial n_i} \right)_{p, T, n_j \neq n_i}$$

where μ_i is the partial molar free energy with respect to the substance i . This is called chemical potential, and it expresses how the free energy of a solution changes when we add or remove an infinitesimal amount of i

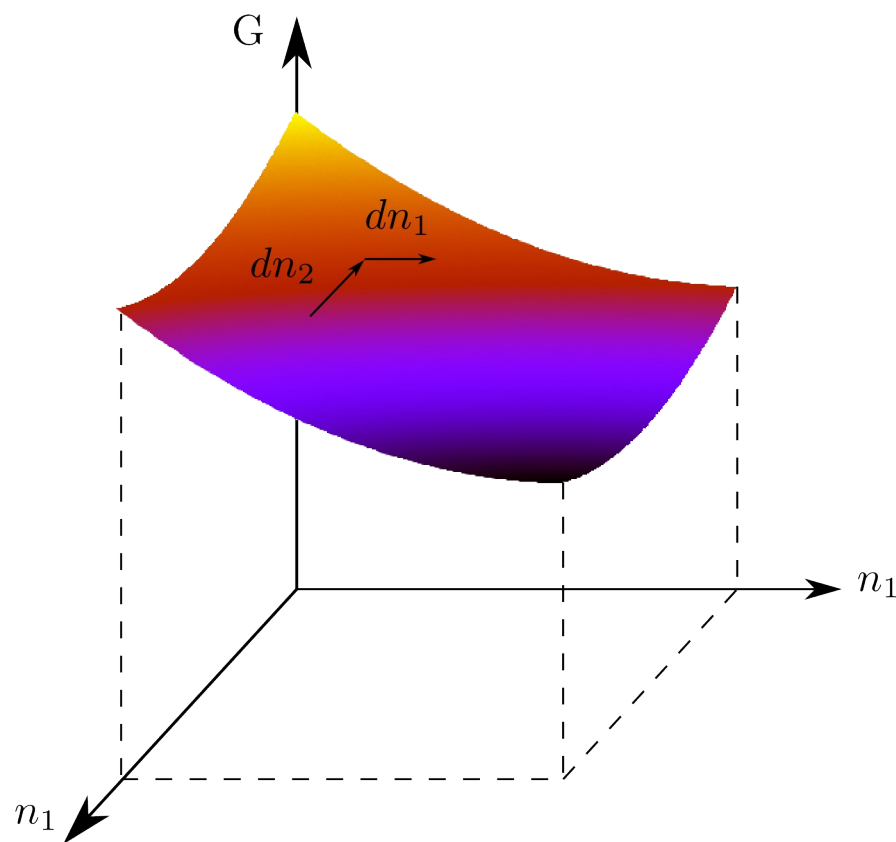
- For simplicity, let us examine a solution of two components
- Let us suppose that we have a solution of urea with free energy G
- If we add a little urea (component 1), we change G
- Similarly, if we add a little water (component 2), we also change G

- If the temperature and the pressure are constant then the change of the free energy is

$$dG = \left(\frac{\partial G}{\partial n_1} \right)_{T, p, n_2} dn_1 + \left(\frac{\partial G}{\partial n_2} \right)_{T, p, n_1} dn_2$$

- This is the fundamental equation of solution thermodynamics

Free energy of an urea solution



- The value of G depends on n_1 and n_2
- This result in a surface in 3D space
- If you vary n_1 , you move along a curve whose projection onto the n_1, n_2 plane is parallel to the n_1 axis
- The slope of this curve at any point n_1 is the partial derivative

$$\left(\frac{\partial G}{\partial n_1} \right)_{T, p, n_2}$$

- Likewise for n_2

$$\left(\frac{\partial G}{\partial n_2} \right)_{T, p, n_1}$$

- The equation for the differential of the free energy can be written in terms of the chemical potentials of the two components

$$dG = \mu_1 dn_1 + \mu_2 dn_2$$

- It is worth noting that μ is a function of temperature, pressure and composition but not of absolute amount
- For example, if two beakers contain different volumes of the same solution, i.e.

$$n_1 \neq n_1' \quad \text{and} \quad n_2 \neq n_2'$$

but

$$n_2/n_1 = n_2'/n_1'$$

then they have the same composition and thus the same *chemical potential*

- Now, let us construct a solution, keeping the above principle in mind
- Let us take an infinitesimal amount of urea and an infinitesimal amount of water and mix them
- The free energy for this infinitesimal amount of solution is

$$\delta G = \mu_1 dn_1 + \mu_2 dn_2$$

- Repeat this 10^6 times and mix all of the little volumes of solutions
- Because chemical potentials have remained constant, you can get the total free energy, G , of the solution by adding up all of δG 's
- This allows us to integrate δG over the whole solution

$$\int \delta G = \mu_1 \int dn_1 + \mu_2 \int dn_2$$

- Performing this integration we get

$$G = \mu_1 n_1 + \mu_2 n_2$$

which is called the *additivity rule*

- Now let us differentiate the additivity rule, taking the total derivative:

$$dG = \mu_1 dn_1 + \mu_2 dn_2 + n_1 d\mu_1 + n_2 d\mu_2$$

- Since we know that

$$dG = \mu_1 dn_1 + \mu_2 dn_2$$

thus

$$n_1 d\mu_1 + n_2 d\mu_2 = 0$$

which is the *Gibbs-Duhem equation*

- The Gibbs-Duhem equation is valuable because if you know one chemical potential you can calculate the other

$$d \mu_2 = - \left(\frac{n_1}{n_2} \right) d \mu_1$$

- Integrating it

$$\int_{\mu_2^0}^{\mu_2} d \mu_2 = - \int_{\mu_1^0}^{\mu_1} \frac{n_1}{n_2} d \mu_1$$

we get

$$\mu_2 - \mu_2^0 = - \int_{\mu_1^0}^{\mu_1} \frac{n_1}{n_2} d\mu_1$$

the *integrated Gibbs-Duhem equation* where μ_2^0 is an integration constant

Pierre Duhem (1861-1916)



Raoult's law

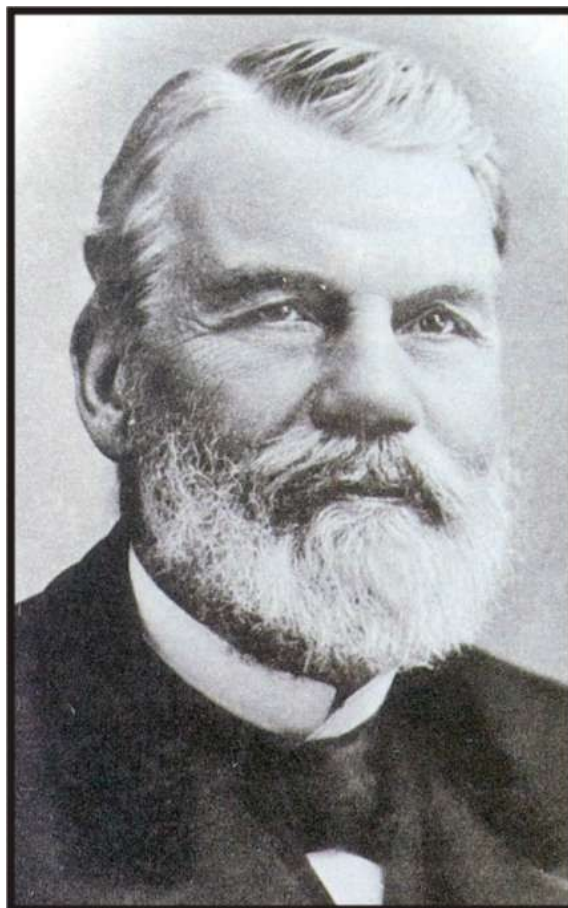
- For an ideal solution, by definition

$$\mu_1 = \mu_1^0 + RT \ln x_1$$

where μ_1^0 is the standard chemical potential and x_1 is the mole fraction of component 1

- This is an empirical relationship that is it was obtained by experiments

Francois-Marie Raoult (1830-1901)



- Raoult's law or the *Law of Dilute Solutions* can also be stated as follows:

$$x_1 = \frac{p_1}{p_1^0} \quad \text{for} \quad x_1 \approx 1$$

i.e. the vapour pressure p_1 of the solvent is proportional to the mole fraction of it

- All real solutions approach this at high dilution
- The “real” equation for μ_1 is

$$\mu_1 = \mu_1^0 + RT \ln \left(\frac{p_1}{p_1^0} \right) = \mu_1^0 + RT \ln x_1$$

since Raoult's law relates the partial pressure of the solvent (p_1/p_1^0) to the mole fraction x_1 of the solvent

- This expression can be used to find μ_2 using the *integrated* form of the *Gibbs-Duhem equation*

$$\frac{d\mu_1}{dx_1} = \frac{d\mu_1^0}{dx_1} + RT \frac{d \ln x_1}{dx_1}$$

- From this we get

$$\frac{d\mu_1}{dx_1} = RT \frac{1}{x_1}$$

$$d\mu_1 = RT \frac{dx_1}{x_1}$$

- Since

$$x_1 + x_2 = 1$$

we can write that

$$dx_1 + dx_2 = 0$$

and after rearrangement

$$dx_1 = -dx_2$$

- The mole fractions of components can be written as

$$x_1 = \frac{n_1}{n_1 + n_2}$$

and

$$x_2 = \frac{n_2}{n_1 + n_2}$$

- Dividing the mole fraction of component 1 by the mole fraction of component 2 we get

$$\frac{x_1}{x_2} = \frac{n_1}{n_1 + n_2} / \frac{n_2}{n_1 + n_2} = \frac{n_1}{n_2}$$

- Let us set out from the *integrated Gibbs-Duhem equation*

$$\mu_2 = \mu_2^0 - \int_{\mu_1^0}^{\mu_1} \frac{n_1}{n_2} d\mu_1$$

- Let us substitute the expression, we obtained for the relationship between mole fractions and amounts of material into the *Gibbs-Duhem equation*

$$\mu_2 = \mu_2^0 - \int \frac{x_1}{x_2} d\mu_1$$

- Further rearrangements can be performed based on the expressions above to get

$$\mu_2 = \mu_2^0 - RT \int \frac{x_1}{x_2} \frac{dx_1}{x_1}$$

and then

$$\mu_2 = \mu_2^0 - RT \int \frac{dx_1}{x_2}$$

- And making use of the equation describing the relationship between the differentials of the two components we get

$$\mu_2 = \mu_2^0 + RT \int \frac{dx_2}{x_2}$$

- Performing the integration, the expression will be

$$\mu_2 = \mu_2^0 + RT \ln x_2$$

- We have now expressions for μ_1 and μ_2 in terms of composition
- These hold for real solutions only when

$$x_1 \approx 1 \quad \text{and} \quad x_2 \approx 0$$

diluted solutions

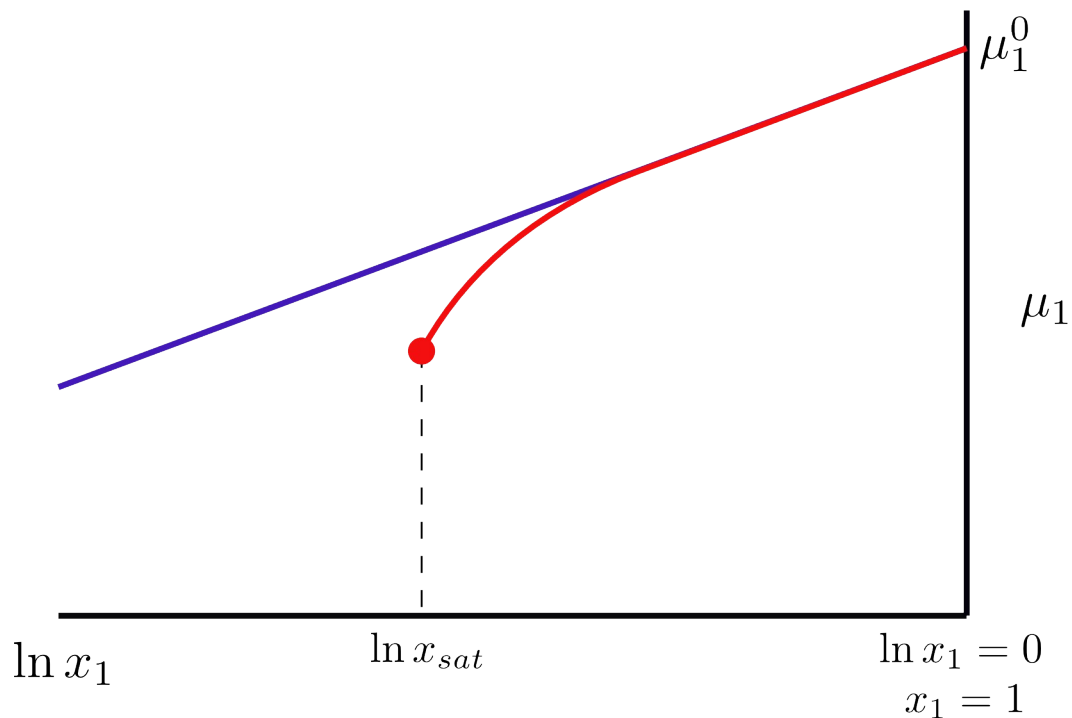
- Some rules of thumb for real solutions approaching ideal solutions
 - Non-ionizable solutes (for example glucose)
 - Ideality up to 0.01-0.1 M
 - Ionizable solutes (for example NaCl)
 - Ideality up to ≈ 0.001 M
 - Proteins
 - Ideality up to 10^{-6} - 10^{-5} M

- Now let us assign numerical values to μ 's
- Let us set out from Raoult's law

$$\mu_1 = \mu_1^0 + RT \ln x_1$$

- Let us plot μ_1 as a function of $\ln x_1$

μ_1 vs. $\ln x_1$



- In the figure, μ_1^0 represents the chemical potential of pure solvent, which means the change in the free energy, G , with addition of pure water and x_{sat} represents the mole fraction of water at saturation
- The purple curve applies to ideal solutions and the red curve applies to real ones where saturation may occur

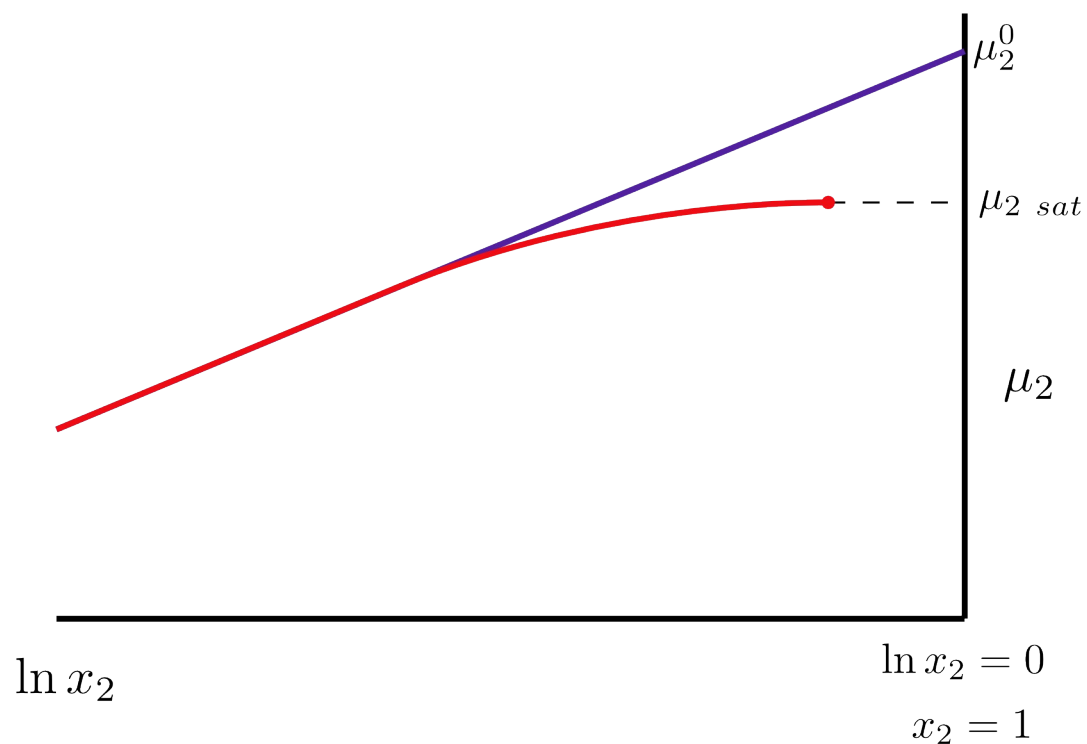
- The ideal law is a limit law
- At low concentrations, the two curves coincide
- Deviation occurs at higher concentration and the “real” curve stops at saturation
- To get the real curve we should plot μ_1 vs. $\ln(p_1/p_1^0)$

- Likewise for the solute

$$\mu_2 = \mu_2^0 + RT \ln x_2$$

- Let us plot μ_2 vs. $\ln x_2$

μ_2 vs. $\ln x_2$



- μ_2^0 has no physical meaning, it is impossible to make solution at this concentration
- The expression for μ_2 is valid over the same region ($x_2 \approx 0$, $x_1 \approx 1$) as the expression for μ_1 since μ_2 was derived from μ_1
- **For any system at equilibrium, the chemical potential of an uncharged substance is the same in all phases between which it can pass**

Henry's law

- It was recognized by *William Henry* that for some solutions, Raoult's law does not hold
- Although the mole fraction of the solute in these solutions continues to be proportional to the vapour pressure, the proportionality constant is not the vapour pressure but a different constant with pressure dimension

- So Henry's law is

$$p_2 = x_2 K_2$$

where p_2 is the vapour pressure of the solute above the solution, x_2 is the mole fraction of the solute and K_2 is a constant of pressure dimension

William Henry (1775-1836)



Activity

- Since measuring the mole fractions of the components of a solution is difficult but measuring the molar concentration is quite simple, biochemists prefer expression containing molar concentrations rather than mole fractions
- To be able to work with such expressions, we have to find the relationship between these two quantities

- We know that in a simple two-component solution:

$$x_2 = \frac{n_2}{n_1 + n_2}$$

where x_2 is the mole fraction of the solute and n_1 and n_2 are the amounts of material of the solvent and the solute, respectively

- We can divide the numerator and denominator by the same factor so that the value of the fraction does not change, thus

$$x_2 = \frac{n_2 / dm^3}{n_1 / dm^3 + n_2 / dm^3} = \frac{c_2}{c_1 + c_2}$$

- Since biochemists usually work with dilute solutions, the concentration of water will remain approximately that of pure water

$$c_{H_2O} \approx \frac{1000 \text{ g} \cdot \text{dm}^{-3}}{18 \text{ g} \cdot \text{mol}^{-1}} \approx 55.56 \text{ M}$$

- For dilute solutions

$$c_2 \ll c_1$$

where c_2 and c_1 are the molar concentrations of solute and solvent, respectively

- Thus

$$x_2 \approx \frac{c_2}{c_1} = \frac{c_2}{55.56 \text{ M}}$$

- Thus, the chemical potential of the solute will be

$$\mu_2 = \mu_2^0 + RT \ln \frac{c_2}{55.56 M}$$

- We can introduce a new constant μ^Δ instead of μ^0

$$\mu^\Delta = \mu^0 - RT \ln 55.56$$

- Thus, the expression for the chemical potential of the solute is

$$\mu_2 = \mu_2^\Delta + RT \ln \frac{c_2}{1 \text{ M}}$$

- Now, let us consider again the plot showing the chemical potential of the solute as a function of its molar concentration
- Let us introduce a new quantity such that it makes the real solution have a straight line on the plot
- This new quantity is called *activity* and denoted by a

$$\ln a = \frac{\mu(\textit{real}) - \mu^{\Delta}(\textit{ideal})}{RT}$$

and after rearrangement

$$\mu(\textit{real}) = \mu^{\Delta}(\textit{ideal}) + RT \ln a$$

- At great dilution

$$\mu_2(\textit{real}) = \mu_2(\textit{ideal})$$

and thus

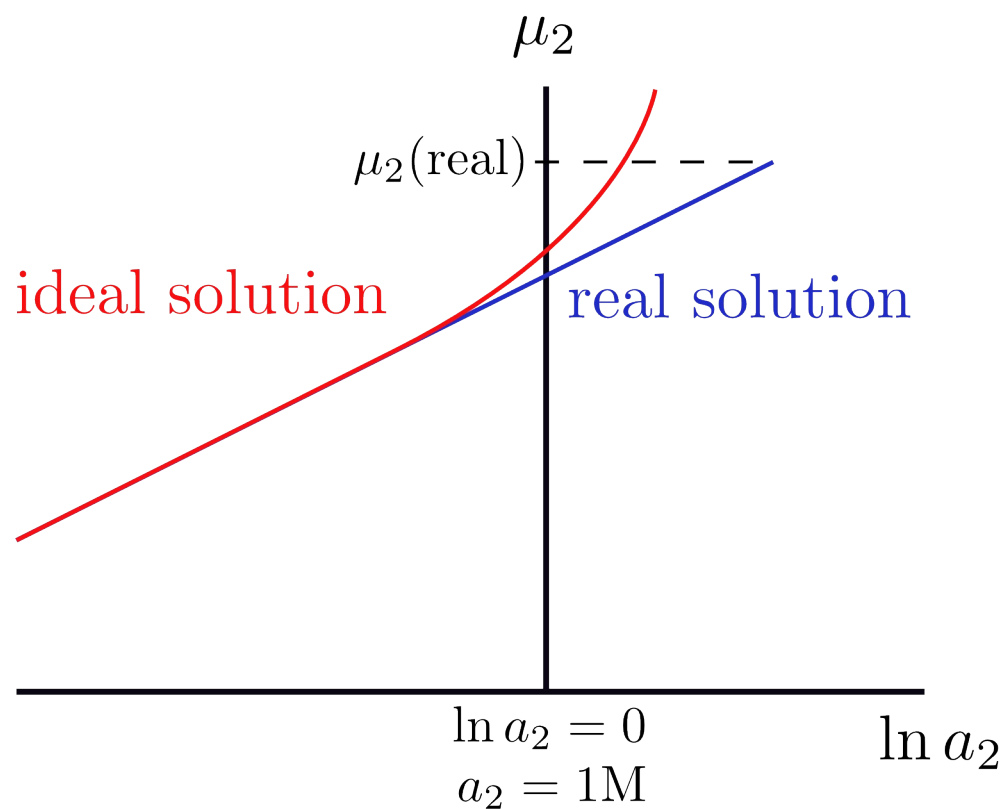
$$a_2 = c_2$$

so

$$\lim_{c_2 \rightarrow 0} a_2 = c_2$$

- If we plot μ_2 vs. $\ln a_2$, the real solution gives a straight line
- The ideal curve now deviates from linearity at higher concentration

μ_2 vs. $\ln a_2$



- Now, however, there is an additional problem: we still do not know how μ (*real*) varies with concentration
- To solve this problem, we define the activity coefficient, which is denoted by γ , such that

$$a_2 = \gamma_2 c_2$$

- The advantage of introducing *activity* and *activity coefficient* is that the laws for real solutions have the same form as the laws for ideal solutions

- The activity has a dimension of concentration
- Because of the definition of the *activity coefficient*, it is unitless
- Furthermore, since

$$a \approx c$$

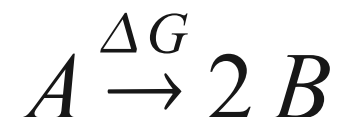
at low concentrations, for the *activity coefficient*

$$\lim_{c \rightarrow 0} \gamma = 1$$

- γ can be obtained from any plot containing an ideal and a real curve
- $RT \cdot \ln \gamma$ is the vertical distance between the two curves at some concentration

Calculating the free energy change of a chemical reaction in solution

- We can relate μ to the change in free energy for a reaction occurring in solution
- For the reaction



what is ΔG when 1 mole of A converts to 2 moles of B?

- We can use the *additivity rule* to get the total G of the whole solution

$$G_{after} = \mu_{H_2O} n_{H_2O} + \mu_A (n_A - 1) + \mu_B (n_B + 2)$$

and

$$G_{before} = \mu_{H_2O} n_{H_2O} + \mu_A n_A + \mu_B n_B$$

- Let us note that since μ is a function of concentration, this subtraction to get ΔG is not straightforward
- As the n 's change so do the μ 's

- To resolve this problem, let us assume that we are working with a huge volume of solution. Then allowing 1 mole of A to convert to 2 moles of B will not noticeably change the composition
- Therefore μ 's before and after are essentially the same
- Alternatively, if we allow only an infinitesimal change of the amount of A, the composition will effectively remain constant

$$\Delta G = G_{after} - G_{before} = 2\mu_B - \mu_A$$

- Substituting

$$\mu_A = \mu_A^0 + RT \ln a_A$$

and

$$\mu_B = \mu_B^0 + RT \ln a_B$$

into the expression above, we get

$$\Delta G = 2 \mu_B^0 - \mu_A^0 + RT \ln \left(\frac{a_B^2}{a_A} \right)$$

where

$$2 \mu_B^0 + \mu_A^0 = \Delta G^0$$

is the *standard reaction free energy*

or expressed in terms of molar concentrations

$$\Delta G = \Delta G^0 + RT \ln \frac{\gamma_B^2 [B]^2}{\gamma_A [A]}$$