

## Supplementary thermodynamics as applied to biosystems

Glucose is transferred to glucose-6-phosphate, abbreviated here to G6P. The reaction may be written



As this process is endergonic (*i. e.*  $\Delta G > 0$ ), it is unnatural (at standard activities). The process is coupled to another process, that of hydrolysis of ATP:



The actual transfer of glucose to G6P is a process, in which both glucose and ATP take place



This process is formally a combination for which we calculate

$$\Delta G^\circ = (+13.8) + (-30.6) = -16.2\text{kJ/mol} \quad (4)$$

which is exergonic (*i. e.*  $\Delta G < 0$ ).

This combination is purely formal, though. If the hydrolysis Eq.(2) took place independently, the free energy available would be lost and no G6P formed, as the process Eq.(1) is endergonic. However, if ATP is molecularly involved in the reactions which are underlying the net process Eq.(1), then the actual, combined process is exergonic as shown.

This kind of calculations (based on standard free energy changes) are often those presented to biology students. However, the actual energetics of such combined processes should be based on free energies calculated for the actual concentrations present, not standard concentrations (1 mol/l). Physiologically realistic values of the concentrations for ATP and ADP are

$$[\text{ATP}] = 1.8 \cdot 10^{-3}\text{mol/l} \quad (5)$$

$$[\text{ADP}] = 1.4 \cdot 10^{-4}\text{mol/l} \quad (6)$$

$$[\text{phosphate}] = 1.0 \cdot 10^{-3}\text{mol/l} \quad (7)$$

and we thus have for Eq.(2)

$$\Delta G = \Delta G^\circ + RT \ln \frac{[\text{ADP}][\text{phosphate}]}{[\text{ATP}]} \quad (8)$$

$$= -30.6 - 23.4 \quad (9)$$

$$= -54.0 \text{ kJ/mol} \quad (10)$$

which is almost double the value based on standard free energies. For comparison,  $\Delta H$  for Eq.(2) with intracellular concentrations is about -18 kJ/mol. Thus a large part of the free energy in this system is entropy determined.

## Thermodynamics of Hydrophobic Interactions.

Some intermolecular forces are intuitively easy to understand. Ion-ion interactions in dissolved salts, and dipole interactions from, say, carbonyl groups, are examples. An important class of interactions arises from hydrogen-bonding, where H bound to fluor, oxygen or nitrogen may bind loosely to another F, O or N. Such hydrophilic forces account for the water solubility of organic compounds with groups like -OH, -COOH, -NH<sub>2</sub>.

The so-called hydrophobic effect is in contrast less obvious. In the lipids consisting of esters of glycerol with two long-chained fatty acids and a phosphate bound to the last hydroxy group of glycerol, the molecule has a hydrophilic part (the phosphate group), but also two long chains resembling alkanes (or alkenes). Such molecules may self-organize into micelles, in which a ball is formed with the hydrophilic groups covering the surface in contact with water, and the interior of the ball consists of the long carbon chains. Another possibility is self-organization to a bilayer membrane, like the one encompassing biological cells. Again, the hydrophilic part of the molecules face towards the water, and the interior consists of the long carbon chains, organized in parallel.

The puzzle with the emergence of such structures is that the forces between the long carbon chains are fairly weak and so are the forces between the chains and water. To understand the hydrophobic effect we may study the simple equilibrium



that is, the solubility in water of a substance A.

For the equilibrium constant we have

$$K = \frac{[A(aq)]}{x(A(l))} \quad (12)$$

which for a pure liquid A yields  $x(A(l)) \simeq 1$  and thus the solubility of  $A(aq) = K$ .

We have

$$\ln(K) = \frac{-\Delta G^\circ}{RT} = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} \quad (13)$$

The solubility is thus dependent on both the enthalpi difference and the difference in entropy for the process. If we try to understand the solubility only in terms of formation and breaking of bonds, or electrostatic interactions, we are largely arguing in terms of the enthalpi difference. For the process  $\text{CH}_3\text{OH}(l) \rightleftharpoons \text{CH}_3\text{OH}(aq)$  one has  $\Delta H^\circ = -7.8\text{kJ/mol}$ . For the corresponding reaction  $\text{CH}_4(l) \rightleftharpoons \text{CH}_4(aq)$  one may derive  $\Delta H^\circ = -8.2\text{kJ/mol}$  that is almost the same. However, for the entropi difference one obtains for methanol  $\Delta S^\circ = +5.0\text{J}/(\text{Kmol})$ , but for methane  $\Delta S^\circ = -64.0\text{J}/(\text{Kmol})$ .

Thus in the case of methane, to create the presumptive solution, the entropy change is toward much higher order. Somehow the entropy change of *water* is negative when we try to introduce methane into it, and this counteracts the solubility process. Carbon chains thus tend to stay together to minimize exposure to water with its corresponding tendency to induce a negative entropy change. This is the hydrophobic effect.

## Electrochemistry as applied to biosystems

In standard courses of chemistry, Nernst's equation for an electrochemical cell is treated. We may thus recall that two redox processes



may occur in an electrochemical cell



with

$$E = E_2 - E_1 \quad (17)$$

$$= E^o - \frac{N}{z} \log Q \quad (18)$$

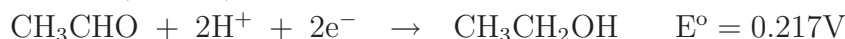
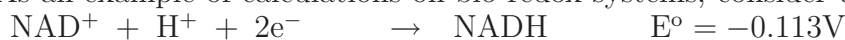
where  $Q$  is the mass-action fraction for the so-called 'cell reaction', *defined* as



and thus

$$Q = \frac{[ox_1][red_2]}{[red_1][ox_2]} \quad (20)$$

As an example of calculations on bio-redox systems, consider the systems



If we want to write a scheme of the form



which has the specific cell reaction



then we observe that  $CH_3CHO$  according to (22) is an ox-form (it is listed on the same side as the electrons) and according to the definition of the cell reaction, Eq(19), the ox-form on the left hand side is  $ox_2$ . Thus the aldehyde/alkohol system is system 2, and our scheme becomes



and we get

$$E = 0.217 - (-0.113) - \frac{N}{2} \log \frac{[NAD^+][CH_3CH_2OH]}{[NADH][CH_3CHO][H^+]} \quad (24)$$

In oxygen consuming biological cells, the so-called respiratory chain is responsible for oxydation of a number of compounds from metabolism. Traditionally, the energetics in the respiratory chain is discussed in standard biochemical text books on the basis of redox

potentials. Thus consider the oxidation of a metabolite such as an aldehyde to an acid, briefly  $\text{OX} + 2e^- \rightarrow \text{RED}$ , coupled to the biochemical redox system  $\text{NAD}^+$ ,  $\text{NADH}$ .

The total process is then



The  $\text{NAD}^+$ ,  $\text{NADH}$  system in turn reacts with a new redox system, with the result that the reduced form  $\text{NADH}$  is oxidized back to  $\text{NAD}^+$ , and this transfer continues through a series of redox systems ('the respiratory chain') until the final reductor is oxidized back by the system  $\text{O}_2, \text{H}_2\text{O}$ . The total process is then an oxidization of  $\text{RED}$  by free oxygen to  $\text{OX}$ .

It is common to discuss the energetics on the basis of the process



We have



We calculate

$$E = E_2 - E_1 - \frac{N}{4} \log \frac{[\text{NAD}^+]^2}{[\text{NADH}]^2 [\text{P}_{\text{O}_2}] [\text{H}^+]^2} \quad (29)$$

which evaluates to  $\simeq 1.14 \text{ V}$  at  $\text{pH} = 7$ , if realistical concentrations are substituted.

For the maximal effective work we have

$$(-W')_{max} = -\Delta G = zFE \quad (30)$$

that is (with  $F = 96500 \text{ C/mol}$ )

$$(-W')_{max} = 4 \times 96.5 \times 1.14 = 440 \text{ kJ/mol} \quad (31)$$

or  $220 \text{ kJ}$  per mole of  $\text{NADH}$ .

To convert  $\text{ADP}$  to  $\text{ATP}$  requires  $50\text{-}60 \text{ kJ/mol}$ , and on a purely energetic basis we thus conclude that some  $220/55 = 4.0 \text{ mol ATP}$  is the maximum which may be produced, if the complete chain operates thermodynamically reversible and the  $\text{ADP}$ ,  $\text{ATP}$  system is molecularly coupled to the redox processes. In the actual biosystem (the membranes of the mitochondria) a total of approx.  $3 \text{ moles of ATP}$  is produced per mole of  $\text{NADH}$  and thus the efficiency is a remarkable  $75 \%$ .

In biochemistry, it is common to use so-called reduced standard potentials,  $E^{\circ'}$ . The idea is that most redox systems in biochemistry depends heavily on  $[\text{H}^+]$ , and as  $\text{pH}$  is around  $7$ ,  $[\text{H}^+]$  is far from the standard state of  $1 \text{ mol/l}$ . Consider a redox system with  $\mathcal{L}$  protons on the left hand side



We have

$$E = E^\circ - \frac{N}{z} \log \frac{[\text{red}]}{[\text{ox}][\text{H}^+]^{\mathcal{L}}} \quad (33)$$

$$= E^\circ - \frac{N}{z} \log \left( \frac{[\text{red}]}{[\text{ox}]} \right) \left( \frac{1}{[\text{H}^+]^{\mathcal{L}}} \right) \quad (34)$$

From the usual rule  $\log(a \times b) = \log(a) + \log(b)$  we may rewrite this as

$$E = E^\circ - \frac{N}{z} \log \frac{1}{[\text{H}^+]^{\mathcal{L}}} - \frac{N}{z} \log \frac{[\text{red}]}{[\text{ox}]} \quad (35)$$

If  $\text{pH} = 7$ , we get

$$\frac{N}{z} \log \frac{1}{10^{-7 \times \mathcal{L}}} = \frac{N}{z} 7 \times \mathcal{L} \quad (36)$$

$$= \frac{\mathcal{L}}{z} \cdot 0.414 \quad (37)$$

Thus Eq(35) evaluates to

$$E = E^{\circ'} - \frac{N}{z} \log \frac{[\text{red}]}{[\text{ox}]} \quad (38)$$

where we have introduced the reduced standard potential  $E^{\circ'}$

$$E^{\circ'} = E^\circ - \frac{\mathcal{L}}{z} \cdot 0.414 \quad (39)$$

A similar calculation, where the protons appear on the right side, say  $\mathcal{R}$  protons, would result in

$$E^{\circ'} = E^\circ - \frac{-\mathcal{R}}{z} \cdot 0.414 \quad (40)$$

In general, with  $\mathcal{L}$  protons on the left side, or  $\mathcal{R}$  protons on the right side, we get

$$E^{\circ'} = E^\circ + \frac{\mathcal{R} - \mathcal{L}}{z} \cdot 0.414 \quad (41)$$

For a redox system, which does not contain protons,  $E^{\circ'} = E^\circ$ .

As an example, consider the above calculations on the process



We find  $E^{\circ'} = -0.113 + \frac{0-1}{2} \cdot 0.414 = -0.320\text{V}$  for the  $\text{NAD}^+/\text{NADH}$  system, and  $E^{\circ'} = 0.815\text{V}$  for the  $\text{O}_2$  system. At  $\text{pH} = 7$  we thus get

$$E = E_2^{\circ'} - E_1^{\circ'} - \frac{N}{4} \log \frac{[\text{NAD}^+]^2}{[\text{NADH}]^2 \text{P}_{\text{O}_2}} \quad (43)$$

Analogously a reduced standard state may be introduced for free energy calculations. For a chemical reaction



we have

$$\Delta G = \Delta G^\circ + RT \ln \left( \frac{[\text{B}]^b}{[\text{A}]^a [\text{H}^+]^{\mathcal{L}}} \right) \quad (45)$$

$$= \Delta G^{\circ'} + RT \ln \left( \frac{[\text{B}]^b}{[\text{A}]^a} \right) \quad (46)$$

with

$$\Delta G^{o'} = \Delta G^o + RT \ln \left( \frac{1}{[\text{H}^+]\mathcal{L}} \right) \quad (47)$$

which in general, with  $\text{pH} = 7$  and  $25^\circ\text{C}$ , evaluates to

$$\Delta G^{o'} = \Delta G^o + (\mathcal{L} - \mathcal{R}) 7 RT \ln(10) \quad (48)$$

$$= \Delta G^o + (\mathcal{L} - \mathcal{R}) 39.93 \text{ kJ/mol} \quad (49)$$

Alternatively one may calculate  $\Delta G^{o'}$  by

$$\Delta G^{o'} = -zF(E_2^{o'} - E_1^{o'}) \quad (50)$$

## Osmotic work

We shall continue with some remarks about so-called osmotic work. For transport of substances and ions over biological membranes, the free energy change corresponding to the transfer of an uncharged substance from, say, the inside of a biological cell to the outside, with concentrations  $c_i$  and  $c_o$  respectively, is

$$\Delta G = RT \ln \frac{c_o}{c_i} \quad (51)$$

and the corresponding effective work is occasionally referred to as the osmotic work connected to the process. For the transport of the ion  $\text{Na}^+$  out of a biological cell, we have additionally to take any electrical potential difference in account

$$W' = RT \ln \frac{c_o}{c_i} + zF(\phi_o - \phi_i) \quad (52)$$

per mole. Typical values are  $c_o/c_i \simeq 12 > 0$  and  $\Delta\phi = +90\text{mV}$ . This process is thus not natural, but energetically uphill, and in the actual cell the process is coupled to the ATP, ADP system.

Transport of ions over membranes in the presence of an electrochemical gradient is common. This may result in ATP creation, or the process may require ATP hydrolysis, in which case we have active transport. The processes in mitochondria, described by Mitchell's chemiosmotic model, are important examples, as we will illustrate with an example.

Living cells are capable of exploiting the free energy difference due to dilution (osmotic work). Consider the process



Suppose  $c_2 < c_1$ . The process is thus a transfer of  $A$  from a dense to a thin solution, which is a natural process. However, the process is connected to a fall in free energy, and this may drive synthesis of, say,  $ATP$  from  $ADP$ , if these substances are coupled to the transport system, allowing  $A$  to move from (1) to (2). However, calculations show that the concentration ratio has to be very high.

If  $A$  is the kation  $H^+$ , and a membrane potential is present as well,  $\Delta\phi = \phi_2 - \phi_1 = -90\text{mV}$ , it may be calculated that the ratio  $c_1/c_2$  now may be substantially smaller to provide enough free energy for the synthesis of one mole of  $ATP$ . The ratio of  $H^+$  to  $ATP$  in the actual mechanism for  $ATP$  synthesis from protons does not need to be one.

If the transfer of 3 moles of  $H^+$  is required for the synthesis of 1 mole of  $ATP$ , it may be calculated that the ratio  $c_1/c_2$  now becomes within range of realistic values of biosystems.

A laboratory use of Eq.(55) is worth mentioning. Some artificial membranes are ion selective, that is, only a certain ion is able to transfer between the two halfcells. If such a membrane is placed between two solutions with different concentration, the ion moves from the dense toward the thinner solution. As it is the only ion which moves through the membrane however, an electrical potential between the two solutions emerges, which counters this transport, until an equilibrium is reached. From Eq.(52) we may calculate this membrane potential:

$$W' = RT \ln \frac{c_2}{c_1} + zF(\phi_2 - \phi_1) = 0 \quad (54)$$

from which we find

$$\Delta\phi = -\frac{RT}{zF} \ln \frac{c_2}{c_1} = -\frac{N}{z} \log \frac{c_2}{c_1} \quad (55)$$

Note that the sign of the ion transferred is relevant here. An ion selective, anion permeable, membrane would yield a membrane potential where  $z$  in Eq.(55) is negative.

Now, consider an electrochemical cell of the form



There is no net 'cell reaction' at the two Pt electrodes, and thus  $E_2 - E_1$  calculated from Nernst equation is zero.

$E = \Delta\phi = \phi_2 - \phi_1$ , where  $\Delta\phi$  is the equilibrium membrane potential found above. The equation Eq.(55) is thus extensively used as a basis for *ion selective electrode*, such as  $H^+$ ,  $Na^+$ ,  $K^+$ ,  $Ca^{2+}$ ,  $Cl^-$ , etc. electrodes, which play an important role in practical laboratory work. Such electrodes may be refined to have dimensions small enough to be inserted in single biological cells.

## Self-organization in (bio)chemical systems.

Biosystems are characterized by a number of processes which intuitively may be perceived as running spontaneously toward higher order. On closer examination, it turns out that many such processes are perfectly in accordance with the laws of thermodynamics, and thus such self-organization is compatible with physics and chemistry.

As an example of self-organization which falls well within physical chemistry, consider the spontaneous formation of crystals out of a supersaturated solution. Thus  $Ag^+$  and  $Cl^-$  may form  $AgCl(c)$ , if a solution of a soluble silver salt is poured into a solution containing sufficient chloride ions. In the precipitate formed, silverchloride crystals consist of a highly ordered lattice of alternating silver and chloride ions. Thus a highly ordered structure forms spontaneously.

The energetics of the process may be discussed from data in the following table.

Species	$\frac{\Delta H_f^\circ}{\text{kJ/mol}}$	$\frac{S^\circ}{\text{J/(Kmol)}}$
$Ag^+$	106	96
$Cl^-$	-167	-122
$AgCl(c)$	-127	-57

For the process



we thus get  $\Delta S^\circ = (-57) - [(-122) + 96] = -31\text{J}/(\text{Kmol})$ . Thus as expected, the process has a negative  $\Delta S^\circ$  and self-organization to higher order occurs when the process takes place spontaneously.

To check that the process is indeed natural, we calculate

$$\Delta H^\circ = (-127) - [(106) + (-167)] = -66\text{kJ/mol} \quad (58)$$

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ = -66 - 298.2 \times (-31 \cdot 10^{-3}) = -56.8\text{kJ/mol} < 0 \quad (59)$$

which is negative. At constant temperature and pressure, the crystallization process is thus 'downhill' in terms of free energy. In this case, the fall in enthalpy is large enough to create a spontaneous process, even though the process is running towards greater order ( $\Delta S^\circ < 0$ ).

We may further note that  $\Delta H^\circ = Q + W' = Q = -66\text{kJ/mol}$  and since  $Q$  is negative,  $66\text{kJ/mol}$  is given off as heat to the surroundings. Since we keep temperature constant, this means that we transfer an entropy amount of  $|Q|/T = 66 \times 10^3/298.2 = 221\text{J}/(\text{Kmol})$  to the surroundings. This is a far greater increase in disorder than the created order ( $31 \text{ J/K mol}$ ) in the crystallization process, so altogether a substantial increase in disorder has happened in the system *plus surroundings*.

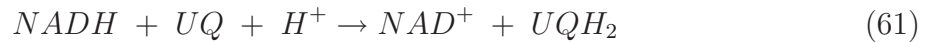
These simple calculations emphasize that it is perfectly possible to increase order spontaneously within a subsystem, if the subsystem plus surroundings experience an increase in disorder. Self-organization in biosystems may thus also be possible spontaneously.



**Exercise 1** In the respiratory chain, the  $NAD^+$ ,  $NADH$  system is coupled to the system ubiquinon, dihydroubiquinon, ( $UQ$ ,  $UQH_2$ ) through intermediary redox systems.



Consider the reaction



a) Write up a formal electrochemical cell, for which (61) would be the 'cell reaction'.  
 b) Find  $E$  and  $\Delta G$  for this cell reaction for actual activities corresponding to the following measured values:

78 % of the  $NAD^+$ ,  $NADH$  system is on the  $NADH$  form.

64 % of the  $UQ$ ,  $UQH_2$  system is on the  $UQ$  form.

(assume  $pH = 7$ ).

c) On the assumption that the generation of ATP is directly linked to the above redox process, how many moles of ATP may this total process produce per mole of  $NADH$ ?

(Note: Actually, the redox process is indirectly coupled to the ATP synthesis. The redox process produces a transport of  $H^+$  ions out over the membrane. On return, the  $H^+$  ions are linked to the synthesis of ATP. See exercise (2)).

d) Calculate  $E_i^{\circ'}$  for both redox systems,  $E^{\circ'} = E_2^{\circ'} - E_1^{\circ'}$  and  $\Delta G^{\circ'}$  for the cell reaction.

**Exercise 2** Living cells are capable of exploiting the free energy difference due to dilution (osmotic work). Consider the process



Suppose  $c_2 < c_1$ . The process is thus a transfer of  $A$  from a dense to a thin solution, which is a natural process. However, the process is connected to a fall in free energy, and this may drive synthesis of, say, ATP from ADP, if these substances are coupled to the transport system, allowing  $A$  to move from (1) to (2).

a) How large must the ratio  $c_1/c_2$  be to provide enough free energy for the synthesis of one mole of ATP?

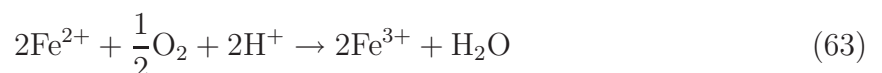
b) If  $A$  is the cation  $H^+$ , and  $\Delta\phi = \phi_2 - \phi_1 = -90mV$ , how large must the ratio  $c_1/c_2$  be now to provide enough free energy for the synthesis of one mole of ATP?

c) Reconsider b). If the transfer of 3 moles of  $H^+$  is required for the synthesis of 1 mole of ATP, what must the ratio  $c_1/c_2$  now exceed?

**Exercise 3** The chemolithotroph *Thiobacillus ferrooxidans* has been examined in

W. J. Ingledew et al.,  
*FEMS Microbiology Letters* 2, p 193-197, 1977.

In this cell, the free energy source for ATP syntheses derives from oxidation of  $Fe^{2+}$  to  $Fe^{3+}$  with  $O_2$ . The overall reaction for oxidation per mole of ATP has been found to follow the stoichiometry



a) Find  $E^\circ$  in a table for the processes

$Fe^{3+} + e^- \rightarrow Fe^{2+}$  and  $O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$ . For the elektrochemical cell



we put  $pH$  in the right halfcell equal to 5.5 and  $P_{O_2} = 0.20$  bar. In the left halfcell, the ratio between the total concentrations  $C_{Fe(II)}/C_{Fe(III)} = 0.01$ , but due to complex formation between  $Fe^{3+}$  and sulfate ions, the ratio between actual concentrations is rather

$$\frac{[Fe^{2+}]}{[Fe^{3+}]} = \frac{1.00 \cdot 10^{-2}}{9.0 \cdot 10^{-3}} = 1.11 \quad (65)$$

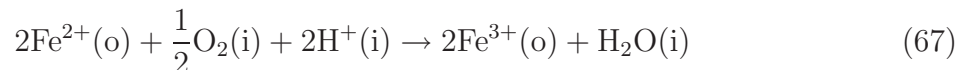
Calculate  $E$  for the electrochemical cell.

For syntheses of ATP we take



Calculate  $\Delta G$  for the process Eq.(63) from  $E$ , and show that this value for  $\Delta G$  is not sufficient for synthesis of one mole of ATP from oxidation of  $2Fe^{2+}$ .

b) The actual mechanism for ATP formation is thus more complicated. Both iron ions are situated outside the membrane system of the bacterium, whereas the oxygen to water reaction takes place inside the membrane system. We thus repeat Eq.(63), but now in the form



That is to say, the process consumes  $2H^+$  on the inside of the membrane. When the oxidation takes place, the inside of the cell thus is deprived of protons. This matches the observation that  $pH$  on the outside is measured to the fairly acidic value  $pH = 2.00$ , but on the inside, as mentioned  $pH = 5.5$ . Moreover, a membrane potential builds up,  $\Delta\phi = \phi(i) - \phi(o) < 0$ . ATP is now created on the inside of the membrane, coupled to the process



when  $2H^+$  is transported back from the outside to the inside. How large a  $\Delta\phi$  is required to ensure that  $W'_{rev}$  in (68) is sufficient to create 1 mol ATP per 2 mol  $H^+$  ?