Biological Oxidation-Reduction Reactions

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http://aris.gusc.lv/BioThermodynamics/OxRedBiologicalW.pdf

The **transfer** of **phosphoryl groups** is a central feature of metabolism. Equally important is another kind of **transfer**, **electron transfer** in **oxidation-reduction**(**OxRed**) reactions. These reactions involve the loss of electrons **-e**$^-$ by one chemical species, which is thereby **oxidized**, and the gain of electrons **+e**$^-$ by another, which is **reduced**. The flow of electrons => **e**$^-$ in **oxidation-reduction** (**OxRed**) reactions is responsible, directly or indirectly, for all work (**W**) done by living organisms. In **non-photo synthetic** organisms, the sources of electrons **e**$^-$ are **reduced compounds** (foods); in **photo synthetic** organisms, the initial electron **-e**$^-$**donor** is a chemical species excited by the absorption **A** of **light ~hν = E energy**. The path of electron **-e**$^-$ flow => in metabolism is complex. Electrons **e**$^-$ move from → various metabolic intermediates to specialized electron **e**$^-$ carriers in **enzyme** - catalyzed reactions. The **carriers e**$^-$ in turn **donate** electrons **e**$^-$ to **acceptors** with higher electron **e**$^-$**affinities**, with the release of **energy**. Cells contain a variety of molecular **energy transducers**, which convert the **energy** of electron **e**$^-$ flow into useful work: **W = E•F•n** ; where,
E potential between species (in volts **V**); **F = 96485 C** (coulomb) **1 mol** of electrons **e**$^-$ electric charge in **C**;
n number of electrons **e**$^-$ involved in species **OxRed** reactions - between **oxidized** state (**Ox)** and **reduced** state (**Red**) of compound. Established so called **OxRed** system with own **RedOx** potential **E** :

$$\mathbf{Ox + ne^- \Leftrightarrow Red\ ;\ E = E^\circ + \frac{\ln(10) \bullet R \bullet T}{F \bullet n} \bullet \lg\left(\frac{[Ox]}{[Red]}\right)} \qquad (1)$$

where **E°** - standard potential of given **OxRed** system measured at conditions when **E = E°** (as **[Ox] = [Red]**); natural logarithm of number **10** - **ln(10) = 2.302585093** ; universal gas constant - **R = 8.3144 J/mol/K** ; absolute thermodynamics temperature **T = 273.16° + 25° C = 298.16° K** at standard temperature conditions measured : as Kelvin scale value **273.16 K** at zero **0°C** point plus on Celsius scalemeasured**25°C** but human body temperature **37° C** that will be higher **T = 273.16° + 37°** (**C**) **= 310.16° K** non-standard conditions;
Faraday's constant - **F = 96 485 C** (coulomb) **1 mol** of electrons **e**$^-$ electric charge in **C**;
number of electrons **e**$^-$ involved in **OxRed** system - **n** ; decimal logarithmic function - **lg()** of argument as ratio **([Ox]/[Red])** between **oxidized form** concentration - **[Ox]** as multiple over **reduced form** concentration **[Red]**.

We begin our discussion with a description of the general types of metabolic reactions in which electrons **e**$^-$ are **transferred**. After considering the theoretical and experimental basis for measuring the **energy** changes **ΔG** in **oxidation** reactions in terms of **electromotive force** (**EMF**), we discuss the relationship between this **force**, expressed in volts **V**, and the **free-energy** change **ΔG** , expressed in joules **J**. We conclude by describing the structures and **oxidation-reduction** chemistry of the most common of the specialized electron **e**$^-$ carriers, which you will encounter repeatedly in later discussions.

The Flow of Electrons can do Biological Work

Every time we use a motor, an electric light or heater, or a spark to ignite gasoline in a car engine, we use the flow of electrons **e**$^-$ to accomplish work **W=E•F•n**, where **n** (in units **mol**) is the **electron number** of **moles** moving from **reduced** form **Red** to **oxidized** form **Ox** . In the circuit that powers a motor, the source of electrons **e**$^-$ can be a **battery** containing two **2** chemical species that differ in **affinity** for electrons **e**$^-$. Electrical wires provide a **pathway** for electron **e**$^-$ flow from the chemical species **reduced formRed_1** at one pole(-) of the **battery**, through the motor, to the chemical species **oxidized form Ox_2** at the other second pole(+) of the **battery**. **(-)$Red_1 \Leftrightarrow Ox_1$+ ne**$^-$Electrical transfer **n** number of electrons **ne**$^-$ flow to**Ox_2+ne**$^- \Leftrightarrow$**Red_2(+)** as **electron-carriers** in **biological Ox-Red systems** transfer **n** number of electrons **ne**$^-$flow from **E_1** to**E_2** for closed circuit **electric-motion force** is calculated as difference **$EMF = E_1 - E_2$** in volts **V** .
Because the two **2** chemical species differ in their **affinity** difference for electrons **e**$^-$, electrons **e**$^-$ flow spontaneously through the circuit, driven by a **force** proportional to the difference in electron **e**$^-$**affinity**, the **electro-motive force** (**EMF**). The **electromotive force** (typically a few volts **±1÷3.5 V**) can accomplish work **W=EMF•F•n** if an appropriate **energy transducer** in this case a motor is placed in the circuit. The motor can be coupled to a variety of mechanical devices to accomplish useful work **W = EMF•F•n**.

Living cells have an analogous biological "circuit," with a relatively **reduced** compound such as **glucose** as the source of electrons **e**$^-$. As **glucose** is **enzymatic oxidized**, the electrons **e**$^-$ released flow spontaneously through a series of **electron-carrier** intermediates to another chemical species, such as O_2.
This electron **e**$^-$ flow is **exoergonic** because O_2 has a higher affinity for electrons **e**$^-$ than do the **electron- -carrier** intermediates. The resulting **electro-motive force** provides **energy** to a variety of molecular **energy transducers** (**enzymes** and other proteins) that do biological work **W**. In the mitochondrion, for example, **membrane-bound enzymes** couple electron **e**$^-$ flow to the production of a **trans-membrane pH** difference, accomplishing **osmotic** and **electrical work**. The proton **H**$^+$ gradient thus formed has **potential energy**, sometimes called the **proton-motive force** by analogy with **electro-motive force**. Another **enzyme**, **ATP synthase** in the inner mitochondrial membrane, uses the **proton-motive force** $\mathbf{E_{membrane}}$ to do chemical work **W**: synthesis of **ATP** from **ADP** and **Pi** as protons **H**$^+$ flow spontaneously across the membrane. Similarly, membrane-localized **enzymes** in *E. coli* convert **electro-motive force** to **proton-motive force**, which is then used to power flagella motion.

The principles of electrochemistry that govern energy changes in the macroscopic circuit with a motor and **battery** apply with equal validity to the molecular processes accompanying electron **e**$^-$ flow in living cells. We turn now to a discussion of those principles.

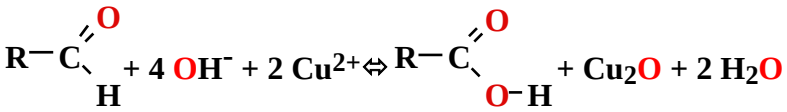
Oxidation-Reductions Can Be Described as Half-Reactions of two Ox ⇔Red Systems

Although **oxidation** and **reduction** must occur together, it is convenient when describing electron **e**$^-$transfers to consider the two halves (**2OxRed** systems) of an **oxidation-reduction** reaction separately. For example, the **oxidation** of ferrous ion **Fe**$^{2+}$ by cupric ion **Cu**$^{2+}$,

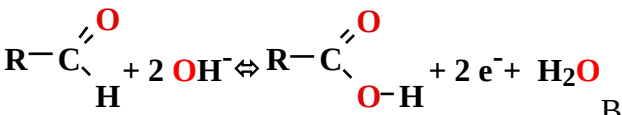
Fe^{2+} **+ Cu**$^{2+}$ => **Fe**$^{3+}$ **+ Cu**$^+$ can be described in terms of two **2** half-reactions (**Ox ⇔Red** systems):

(**1**) **Fe**$^{2+}$⇔ **Fe**$^{3+}$ **+ e**$^-$ The electron-donating **e**$^-$ molecule in an **oxidation-reduction** reaction is called
(**2**) **Cu**$^{2+}$ **+ e**$^-$⇔ **Cu**$^+$ the **reducing** agent or **reductant**; the electron-accepting **+ e**$^-$ molecule is the **oxidizing** agent or **oxidant**. A given agent, such as an iron cation existing in the ferrous (**Fe**$^{2+}$) or ferric (**Fe**$^{3+}$) state, functions as a conjugate **reductant-oxidant** pair (**RedOx** pair), just as an **acid** and corresponding **base** function as a conjugate **acid-base** pair. Recall from **Acid-Base** Equilibrium that in **acid-base** reactions we can write a general equation: proton **donor**⇔**H**$^+$**+** proton **acceptor**. In **RedOx** reactions we can write a similar general equation: electron **donor**⇔**ne**$^-$ + electron **acceptor**. In the reversible half reaction (1) above, **Fe**$^{2+}$ is the electron **donor** and (2) **Fe**$^{3+}$ is the electron **acceptor;** together, **Fe**$^{2+}$ and **Fe**$^{3+}$ constitute a **conjugate RedOx pair.**

The electron **e-** transfers in the **oxidation-reduction** reactions of organic compounds are not fundamentally different from those of inorganic species. In Reducing Sugars we considered the **oxidation** of a **reducing sugar** (an free aldehyde or ketone) by cupric ion **Cu**$^{2+}$(see **reducing sugars**):



This overall reaction can be expressed as two **2** half-reactions using **OxRed** systems:

(1) 

(2) **2 Cu**$^{2+}$ **+ 2 e**$^-$ **+ 2 OH- ⇔ Cu**$_2$**O + H**$_2$**O**

Because two **2** electrons **2 e**$^-$ are removed from the aldehyde carbon **—(C=O)—H**, the second half-reaction (the one-electron reduction of cupric **Cu**$^{2+}$ to cuprous ion **Cu**$^+$) must be doubled **2** to balance the overall equation two **2** electrons **2 e**$^-$ are gained on two cupric **Cu**$^{2+}$ cations converting to two cuprous ions **Cu**$^+$.

Biological Oxidations Often Involve Dehydrogenation

The carbon in living cells exists in a range of **oxidation** states (Fig. 1). When a carbon atom shares an electron pair **:** with another atom (typically **H**, **C**, **S**, **N**, or **O**), the sharing is unequal in favor of the more **electronegative** atom. The order of increasing **electro negativity**: **H < C < S <N<O** respectively

2.2 < 2.55 < 2.58 < 3.04 < 3.44.

In over simplified but useful terms, the more **electronegative** atom "owns" the bonding electrons **e⁻** it shares with another atom. For example, in methane (**CH_4**) carbon **C** is more **electronegative** than the four **4** hydrogens **H** bonded to it, and the **C** atom therefore "owns" all eight **8** bonding electrons **8 e⁻** (Fig. 1). In **ethane**, the electrons **e⁻** in the **≡C-C≡** bond are shared equally, so each **:::C:C:::** atom owns only seven **7** of its eight **8** bonding electrons **e⁻**. In **ethanol**, **C-1** is less electronegative than the oxygen **O** to which it is bonded, and the **O** atom therefore "owns" both electrons **2 e⁻** of the **≡C-:O-** bond, leaving **::˙C-1** with only five **5** bonding electrons **e⁻**. With each formal loss of electrons **e⁻**, the carbon **C** atom has undergone **oxidation** even when no oxygen **O** is involved, as in the conversion of an **alkene** (**CH_2=CH_2**) to an **alkyne** (**CH≡CH**). In this case, **oxidation** (loss of electrons **e⁻**) is coincident with the loss of hydrogen **- H**. In biological systems, **oxidation** is often synonymous, with **dehydrogenation,** and many **enzymes** that catalyze **oxidation** reactions are **dehydrogenases**. Notice that the more **reduced** compounds in Figure 1 (top) are richer in hydrogen **H** than in oxygen **O**, whereas the more **oxidized** compounds (bottom) have more **oxygen O** and less **hydrogen H**.

Not all biological **oxidation-reduction** reactions involve carbon **C**. For example, in the conversion of molecular **nitrogen N_2** to ammonia **NH_3** :**6 H^+ + 6 e⁻ + °N_2=>2 $^{(-3)}NH_3$**, the **nitrogen N** atoms are **reduced**.

Electrons **e⁻**are transferred from one molecule (electron **e⁻donor)** to another (electron **e⁻acceptor)** in one **1** of four **4** different ways:

1. Directly as electrons **e⁻**. For example, the **Fe^{2+} / Fe^{3+}RedOx**pair can transfer an electron **e⁻** to the **Cu^+ / Cu^{2+}RedOx**pair:

Fe^{2+} + Cu^{2+}=>Fe^{3+} + Cu^+

2. As **hydrogen H** atoms. Recall that a hydrogen **H** atom consists of a proton **H^+** and a single electron **e⁻.** In this case we can write the general equation:

AH_2=>A + 2 e⁻ + 2 H^+

where**AH_2** is the **hydrogen/electron donor**.

(Do not mistake the above reaction for an acid dissociation; the **H^+** arises from the removal of a hydrogen atom (**H^+ + e⁻**).) **AH_2** and **A** together constitute a conjugate **RedOx** pair (**A / AH_2**), which can **reduce** another compound **B** (or **RedOx** pair, **B / BH_2**) by transfer of hydrogen **H** atoms:

AH_2 + B =>A + BH_2

3. As a **hydride ion (:H^-)**, which has two **2** electrons **e⁻**.

This occurs m the case of **NAD-linked dehydrogenases**, described below.

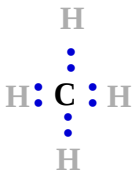
4. Through direct combination with **oxygenO_2**. In this case, oxygen**O_2** combines with an **organic reductant** and is **covalently incorporated** in the product, as in the **oxidation** of a hydrocarbon to an alcohol by **1/2 O_2** presented as **O**:

R-CH_2-H + .:O:. =>R-CH_2-::O::-H

The **hydrocarbon** is the electron **e⁻donor** and the oxygen**O** atom is the electron **e⁻acceptor**.

All four **4** types of electron **e⁻** transfer occur in cells. The neutral term **reducing equivalent** is commonly used to designate a single **1** electron **e⁻ equivalent** participating in an **oxidation-reduction** reaction, no matter whether this **equivalent** is an electron **e⁻** per se, a hydrogen **H** (**H^+ + e⁻**) atom, or a hydride ion **:H^-**, or whether the electron **e⁻** transfer takes place in a reaction with **oxygen O** to yield an **oxygenated** product. Because biological **fuel** molecules are usually **enzymatic dehydrogenated** to lose two **2reducing equivalents** at a time, and because each **oxygen O** atom can **accept** two **2reducing equivalents**, biochemists by convention regard the unit of biological **oxidations** as two **2 reducing equivalents** passing from **substrate**=>to **oxygen O**.

Methane



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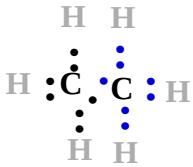
Ethane (alkane)



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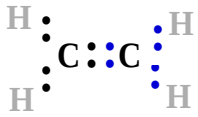
Ethene (alkene)



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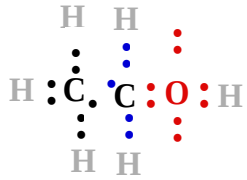
Ethanol **7 5** (alcohol)



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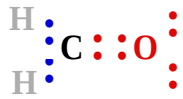
Acetylene (alkyne)

Formaldehyde



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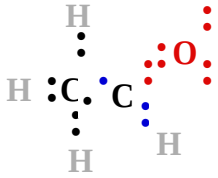
Acetaldehyde (aldehyde) **7 3**



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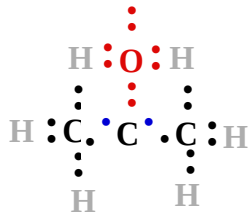
Acetone (ketone) **7 2**



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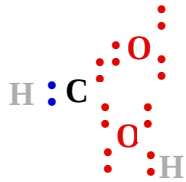
Formic acid



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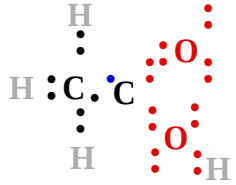
Carbon monoxide

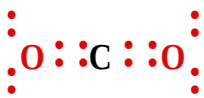
2

Acetic acid (carboxylic **7 1** acid)



1

Carbon dioxide



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7 Figure 1. Oxidation states of carbon **C** occurring in the biosphere.

The **oxidation** states are illustrated with some representative **compounds**. Focus on the black carbon **C** atom and its bonding electrons **e**$^-$.

When this carbon **C** is bonded to the less ↓ **electro negative H** atom, both bonding electrons (blue - : •) are assigned to the carbon **C**.

When carbon **C** is bonded to another carbon **C**, bonding electrons **e**$^-$ are shared equally, so one blue • of the two **2** electrons **e**$^-$**e**$^-$ is assigned to the black carbon **C**.

When the black carbon **C** of our interest is bonded to the more **electronegative O** atom, the bonding electrons **e**$^-$ are assigned to the oxygen **O:**.

The number to the right of each compound is the number **n** of electrons **e**$^-$ "owned" by the black carbon **C** of our interest, a **rough expression** of the **oxidation** state of that carbon **C**.

When the black carbon **C** undergoes **oxidation** (loses electrons **e**$^-$), the number **n**↓ gets smaller.

Thus the order of increasing ↑ **oxidation** state is

methane <
< alkane <
< alkene <
< alcohol <
< alkyne <
< aldehyde <
< ketone <
<formic acid <
<carbon monoxide.
< carboxylic acid <
<carbon dioxide.

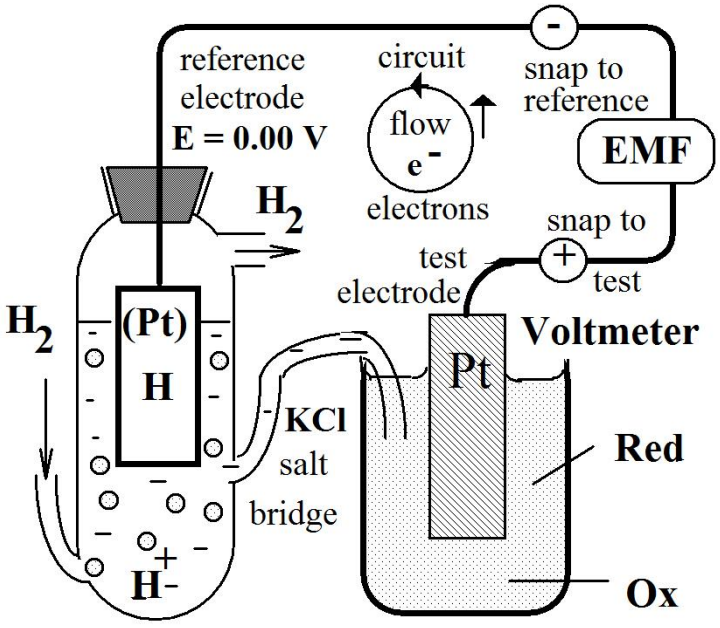
Reduction Potentials Measure Affinity for Electrons

When two **2** conjugate **RedOx** pairs are together in solution, electron **e⁻** transfer from the electron **e⁻donor** of one **RedOx** pair to the electron **e-acceptor** of the other may occur spontaneously. The tendency for such a reaction depends on the relative **affinity** of the electron **e- acceptor** of each **RedOx** pair for electrons **e⁻**.The **standard reduction potential, E°** a measure (in volts **V**) of this **affinity**, can be determined in an experiment such as that described in Figure 2. Electrochemists have chosen as a **standard** of reference the half-reaction
$H^+ + e^- \Leftrightarrow \underline{H}$ **(Pt) ,** ($^1/_2$ H_2) Hydrogen saturated platinum (**Pt**) $H_2$is a solid **metal**- first **I type** electrode.

$$E = E^\circ + \frac{\ln(10) \bullet R \bullet T}{F \bullet 1} \bullet \lg([H_3O^+]) \text{ ; because of Brønsted water protonation } H^+ + H_2O \Rightarrow H_3O^+ \qquad (2)$$

$$E = -0.05916 \bullet pH \text{ ; because } -\lg([H_3O^+]) = pH \text{ , and } \frac{\ln(10) \bullet R \bullet T}{F} = 0.05916\ V \text{ at standard conditions} \qquad (3)$$

The electrode at which this half-reaction occurs (called a half-cell) is arbitrarily assigned a **standard reduction** potential **E°** of **0.00 V**. When this **hydrogen electrode** is connected through an external circuit to another half-cell in which an **oxidized** species and its corresponding **reduced** species are present as pure H_2 compound at standard concentrations **C** (each solute at **1 M,** each gas at **101.3 kPa** or **1 atm**), electrons **e⁻** tend to flow through the external circuit from the half-cell of lower **standard reduction potential** $E°_1$ **t**o the half-cell of higher **standard reduction potential** $E°_2$. By convention, the half-cell with the stronger tendency to acquire electrons **e⁻** is assigned a positive value of $E°_2$>**0.00 V**.



hydrogen electrode pure H_2 gas at standard pressure saturates platinum **Pt** metal with **H hydrogen** forming reference cell with known **E = 0.00V**

Test cell containing **pH=7** concentration of the **oxidized [H^+]** and **reduced H(Pt)** (like solid as left) species of the **RedOx** pair to be examined

Voltmeter the device for measuring **EMF** connected to reference snap and test snap **Figure 2.** Measurement of the **standard reduction potential** (E_o) of a **RedOx** pair at **pH=7** condition. Electrons **e⁻** flow from the **test** electrode to the **reference** electrode if **standard reduction potential** is negative E_o**< 0.00 V** or vice versa if positive E_o**> 0.00 V**. The ultimate **reference** half-cell is the **hydrogen electrode**, as shown here.

Electro-chemists have chosen as a **standard** of reference the half-reaction which this half-reaction occurs is arbitrarily assigned a **standard reduction** potential **E=E°** of **0.00 V pH=0**.

The **electro-motive force** (**EMF=E_o-E**) at **pH=7**, E_o for the **hydrogen electrode RedOx** system $H^+ + e^- \Leftrightarrow \underline{H}\downarrow$**(Pt)**is **-0.41412V=$E_o$=-0.05916•pH** and **EMF=-0.41412-0.0V .** The direction of electron **e⁻** flow → depends on the relative electron "pressure" or **potential** of the two **2** cells. A salt bridge containing a saturated **KCI** solution provides a path for counter-ion movement between the **test** cell and the **reference** cell.

From the observed **EMF** and the known **E = 0.00 V** for **reference** cell, the E_o of the **test** cell containing the **RedOx** pair is obtained as calculation **EMF + E = E_o = -0.41412 V**. The cell that gains electrons has, by convention, the more positive **E** >**E_o reduction potential** and so as well as
E = 0.00 V > -0.41412 V = E_o.

The **reduction potential E** of a half-cell depends not only on the chemical species present but also on their activities, approximated by their concentrations **C**. About a century ago, Walther Nernst derived an equation that relates **standard reduction potential** (**E'**) to the **reduction potential** (**E**) at any concentration of **oxidized [Ox]** and **reduced [Red]** species in the cell:

Red–Ox system tables http://aris.gusc.lv/BioThermodynamics/OxRedBiologicalW.pdf

Half-reaction - OxRed systems	Data source	Eo (V)	EM(V)	E° (V)	E°H_2O(V)	E°37(V)
H_2O_2+2 H_3O^++ 2 e^-= 4 H_2O	Suchotina	1.7356	1.6734	1.776	1.9821	1.9742
O_2^-+2 H_3O^++ e^-= H_2O_2+ 2 H_2O	David Harris	0.305	0.1806	1.2764	1.48246	1.4251
O_{2g}+4 H_3O^++ 4 e^-= 6 H_2O	Suchotina	0.813	0.751	1.2288	1.38334	1.3732
NO_3^-+3H_3O^++2e^-=HNO_2+4H_2O	University Alberta	0.2889	0.1957	0.9275	1.13355	1.1291
NO_3^-+ 2 H_3O^++2e^-= NO_2^-+ 3 H_2O	David Harris	0.3913	0.3291	0.8351	0.98967	0.95138
p-quinone+2H_3O^+ +2e^-=Hydroquinone+2H_2O		0.2336	0.1714	0.6994	0.80243	0.79365
O_{2aq}+2H_3O^++2e^-=H_2O_{2aqua}+2H_2O	University Alberta	0.2336	0.1715	0.6945	0.7975	0.7937
Fe^{3+} + e^- = Fe^{2+}	University Alberta	0.783	0.783	0.7690	0.7690	0.7830
Ubiquinone+2H_3O^+ +2e^-=Ubiquinol+2H_2O		0.0197	0.0819	0.4591	0.56215	0.5404
Fumarate^{2-}+2H_3O^+ +2e^-=Succinate^{2-}+2H_2O		0.0332	0.0953	0.4451	0.54815	0.52695
CrotonylCoA+2H_3O^++2e^-=ButyrylCoA+2H_2O		-0.0774	-0.1395	0.3991	0.50215	0.48273
$C_6H_6O_6$+2H_3O^+ +2e^-=AscorbicAcid+2H_2O	DC.Harris	-.0862	-.1483	0.3900	0.4930	0.47395
Glyoxylate+2H_3O^++2e^-=glycolate+2H_2O	D.C.Harris 25°C	-0.111	-0.171	0.324	0.42715	0.42715
Cytochrome F Fe^{3+} + e^-= Fe^{2+}	David Harris	0.3509	0.3509	0.3650	0.3650	0.3509
$[Fe^{III}(CN)_6]^{3-}$+ e^-= $[Fe^{II}(CN)_6]^{4-}$	University Alberta	0.3258	0.3258	0.3557	0.3557	0.3258
Oxalo-acetate^{2-}+2H_3O^+ +2e^-=Malate^{2-}+2H_2O		-0.2225	-0.2847	0.2481	0.35115	0.33757
Cytochrome a3 Fe^{3+}+ e^-= Fe^{2+}		0.3365	0.3365	0.3500	0.3500	0.3365
Pyruvate$^-$+2H_3O^+ +2e^-=lactate$^-$+2H_2O		-0.2408	-0.3030	0.2291	0.33215	0.3193
FADfree+2H_3O^+ +2e^-=$FADH_2$ +2H_2O	*	-0.2735	-0.3356	0.1951	0.29815	0.28662
CH_3CHO+2H_3O^++2e^-=CH_3CH_2OH+2H_2O	KortlyShucha	-0.2784	-0.3406	0.1900	0.2930	0.28169
Cytochrome a Fe^{3+} +e^-= Fe^{2+}		0.2788	0.2788	0.290	0.290	0.2788
GlutaS-Sthione+2H_3O^+ +2e^-=2GlutathSH+2H_2O		-0.2841	-0.3462	0.1841	0.28715	0.27604
S_{rhb}+2H_3O+ +2e-=HSH+2H_2O	University Alberta	-0.2859	-0.3480	0.1739	0.27693	0.27424
Cytochrome c Fe^{3+} + e^- = Fe^{2+}		0.2442	0.2442	0.254	0.254	0.2442
LipoicAcidS-S+2H_3O^++2e^-=LipSHSH+2H_2O		-0.3417	-0.4039	0.1241	0.22715	0.21837
Cytochrome c1 Fe^{3+} + e^-- = Fe^{2+}		0.2115	0..2115	0.220	0.220	0.2115
AcetoAcetate$^-$+2H_3O^+ +2e^-= β-OHButyrate$^-$+2H_2O		-0.3956	-0.4577	0.0681	0.17115	0.16453
α-Ketoglutarate^{2-}+CO_2+2H_3O^++2e-=isocitrate^{2-}+2H_2O		-0.4283	-0.4904	0.0341	0.13715	0.13185
H_3O^++ e^-=H(Pt) + H_2O		-0.4611	-0.5232	0.000	0.10303	0.09904
Cytochrome b Fe^{3+} + e^- = Fe^{2+}		0.074	0.074	0.077	0.077	0.074
CH_3COOH+2H_3O^++2e^-=CH_3CHO+3H_2O	Suchotina	-0.5784	-0.6407	-0.118	0.03654	0.03513
13PGlycerate^{4-}+ 2H_3O^++2e^-=Glycaldeh3-P^{2-}+2H_2O+Pi^{2-}		-0.5873	-0.6496	-0.1314	-0.0284	-0.0273
$NADP^+$+H_3O^+ +2e^-=NADPH+ H_2O		-0.3429	-0.3740	-0.117	-0.0654	-0.0629
NAD^+ +H_3O^+ +2 e^-=NADH + H_2O	David Harris	-0.3391	-0.3702	-0.113	-0.0614	-0.0590
O2g+ e- = O-2aq	Suchotina	-0.2355	-0.2355	-0.245	-0.245	-0.2355
Ferredoxin Fe^{3+} + e^- = ferredoxin Fe^{2+}		-0.415	-0.415	-0.432	-0.432	-0.415
2$C_3H_4O_3$ + 4H_3O^+ + 4e^- = $C_6H_{12}O_6$ + 4H_2O	Stryer	-.9975	-1.060	-0.5427	-0.4397	-0.4373
H_2O + e^- = H(Pt) + OH^-	Suchotina	-0.5938	-0.6559	-0.828	-0.9311	-0.8951
Half-reaction - OxRed systems	Data source	Eo (V)	EM(V)	E° (V)	E°H_2O(V)	E°37(V)

Table 1. Standard Reduction Potentials E_o and **E_M**of Some Biologically Important Half-Reactions, at **37 °C** for **pH=7.36** and **8.37** (in mitochondria), **E°** at **standard** conditions **298.16 K, pH=0** for **H^+/ H** reference electrode **E°=0.00 V**, **$E°_{H2O}$** corrected to water concentration **[H_2O] = 997.07/18.0153 = 55.3457 M** from equations where involved, and **$E°_{37}$**. at body temperature conditions **310.16 K(37 °C**) calculated from **$E°_{H2O}$**

Data mostly from: 1. Loach, P.A. (I 976) In Handbook of Biochemistry and Molecular Biology,
2. 3rd edn (Fasman, G.D. ed.), Physical and Chemical Data, Vol. 1, pp. 122-130 e, CRC Press,
3. A.M. Suchotina, Handbook of Electro-Chemistry, Petersburg ,1981."Chimia"©
4. S.Kortly and L.Shucha. Handbook of chemical equilibria in analytical chemistry. 1985.EllisHorwood Ltd.©
5. University Alberta Data Tables Molar Thermodynamic Properties of Pure Substances 1997.,
http://www.vhem.ualberta.ca/courses/plambeck/p101/p00403.htm
6. Boca Raton, FL. "This is the value for free **FAD**;
FAD bound to a specific flavo-protein (for example succinate dehydrogenase) has a different **E°**
7. David A. Harris, "Bio-energetic at a Glance". **b** Blackwell Science Ltd ©, 1995, p.116.
8. Daniel C. Harris, "Quantitative chemical analysis". W.H.Freeman and Company ©, 5th ed.1999, p545

$$E = E^{\circ} + \frac{\ln(10) \bullet R \bullet T}{F \bullet n} \bullet \lg\left(\frac{[Ox]}{[Red]}\right)$$

where **E°** - **standard potential** of given **OxRed** system measured at conditions when **E = E°** (as **[Ox]=[Red]**); natural logarithm of number 10 - **ln(10) = 2.302585093** ; universal gas constant - **R = 8.3144 J/mol/K** ; absolute thermodynamics temperature **T = 273.16° + 25°(C) = 298.16° K** at standard temperature **conditions** measured : as Kelvin scale value **273.16 K** at zero **0° C** point plus on Celsius scale measured **25°C** but human body temperature **37°C** that will be higher **T = 273.16° + 37°(C) = 310.16° K** non-standard **conditions**; **Faraday's** constant - **F = 96 485 C** (coulomb) **1 mol** of electrons e^- electric charge in **C**;
At **298 K**(**25 °C**) and at **310.16 K** (**37 °C**), this expression reduces to respectively following expressions:

$$E = E^{\circ} + \frac{0.05916V}{n} \bullet \lg\left(\frac{[Ox]}{[Red]}\right) \; ; \; E = E^{\circ} + \bullet \lg\frac{0.0615V}{n} \bullet \lg\left(\frac{[Ox]}{[Red]}\right)$$

Many half-reactions of interest to biochemists involve protons H^+ or thermodynamically corrected reality **hydronium ion** H_3O^+. As in the definition of **ΔG°**. biochemists define the **standard** state for **oxidation-reduction** reactions as **pH 7.36** and express the **standard reduction potential** as E_o, the standard reduction potential at **pH 7.36**. The **standard reduction potentials** given in Table 1 and used throughout this book are values for E_o and are therefore only valid for systems at neutral **pH**. Each value represents the potential difference when the conjugate **RedOx** pair, at **equal** concentrations **[Ox] = [Red]** and **pH = 7.36**, is connected with the **standard** (**pH=0**) **hydrogen electrode**. Notice in Table 1 that when the conjugate pair H^+/ **H** at **pH 7** is connected with the **standard hydrogen electrode** (**pH 0**), electrons e^- tend to flow from the **pH 7** cell => to => the **standard** (**pH 0**) cell; the measured E_o for the H^+/ **H** pair according (3) is **- 0.41412 V**

Standard Reduction Potentials Can Be Used to Calculate the Free-Energy Change

The usefulness of **reduction potentials** stems from the fact that when **reduction potentials** E_1 and E_2 values have been determined for any two **2**half-cells, relative to the **standard hydrogen electrode potential E°=0.00 V** as reference, their **EMF=**E_{Ox2}**-**E_{Red1}values relative to each other are also known. 'We can then predict the direction Red_1=>Ox_2 in which electrons e^- will tend to flow when the two **2** half-cells are connected through an external circuit or when components Red_1=>Ox_2 of both half-cells are present in the same solution. Electrons e^-tend to flow to the half-cell with the more positive **E°** and the strength of that tendency is proportional to the difference in **reduction potentials**, **ΔE°**.

The energy **ΔG** made available by this spontaneous electron e^- flow by **work** action **ΔG = W** (the **free-energy** change **ΔG** for the **oxidation-reduction** reaction) is proportional ~ to **ΔE**. According **RedOx** system positive charged **oxidized** form Ox^{n+} - formed with lost electrons ne^- - flows from metal site (mostly used platinum **Pt** and bearing free electron ne^- gas) to solution. In this movement process **RedOx** system are accomplished the chemical work **W = E•F•n** by spending given **RedOx** system **free energy** $-\Delta G = -(G_{Ox} - G_{Red})$ in conversion of **reduced** form **Red** to **oxidized** form Ox^{n+}:

$$Red \Leftrightarrow Ox^{n+} + ne^-; \; W = E \bullet F \bullet n = \Delta G \qquad (4)$$

Here **n** represents the number of electrons ne^- transferred in the reaction.

Chemical Potential of Species μ

Professor Ilya Prigogine and co has shown that **chemical potentialμ** of compound **A** show, how much change of **free energy** ΔG_A brings into system of our interest when adding of **1 mol** amount of compound **A** .
In a fact: how great amount of free energy belongs to one **1 mol** of compound.
It means how much free energy ΔG_A has itself per **1 mol** compound **A** ,
– **chemical potential μ** of compound **A** if amount of compound in molar numbers is Δn_A **= 1 mol**

$\mu_A = \frac{\Delta G_A}{\Delta n_A}$; $\mu_A = \Delta G^{\circ} + R \bullet T \bullet \ln(X_A)$, where X_A is concentration of **A** unit less **mol fraction** $X_A = \frac{n_A}{n_{total}}$ (5)

For pure compound **A** when $n_A = n_{total}$ **mol fraction** is X_A **= 1** so **ln(1) = 0** and **μ = ΔG°** that present **standard free energy** of formation the **1 mol** compound **A** from elements.
Reaction proceeds completely until end only when products of reaction have hardly little disposition to reverse change back. In other words these products of reaction have trifling or zero value of **chemical potential**.
Data from: I. Prigogine, R. Defey. "Chemical Thermodynamics".1954, Longmans Green and co ©.

Conditions of chemical equilibrium.

Provided **chemical potential** of reaction products is taking into consideration (it has anything remarkable level of value) , then reaction proceeds not completely until end, go not on but one can observe the setting in **equilibria**. In state of **equilibria** sum of **chemical potentials** for initial compounds is equal to sum of **chemical potentials** for products – according chemical equation

$$\mathbf{Red} \underset{\text{revers}}{\overset{\text{direct}}{\rightleftarrows}} \mathbf{Ox^{n+} + ne^-; W = E \bullet F \bullet n = \Delta G}$$

initial compounds products work accomplished by movement of positively (**n+**) charged $\mathbf{Ox^{n+}}$ form from metal surface, comprising electron $\mathbf{ne^-}$ gas , and touching **Red** form, to solution of **RedOx** system.
For **RedOx** system due to electric work of charged $\mathbf{Ox^{n+}}$ movement between metal and **RedOx** system solution sides are not equal $\mu_{Red} \neq \mu_{Ox^{n+}} + \mathbf{n}\ \mu_{e^-}$ but coefficient $\mathbf{ne^-}$ means $\mathbf{e^- + e^- + e^- \ldots n}$ times electron $\mathbf{e^-}$ takes a part in reaction as is seen in expression of **equilibrium** (4). Free energy change $\Delta\mathbf{G}$ for any chemical reaction is to calculate as **chemical potential** sum subtraction: the product $\Sigma\mu_{product}$ minus initial $\Sigma\mu_{initial}$:
$\Delta\mathbf{G} = (\Sigma\boldsymbol{\mu}_{Ox^{n+}} + \mathbf{n}\ \mu_{e^-}) - \Sigma\mu_{Red} = \mathbf{E \bullet F \bullet n}$, and **equilibrium** establishes when electric work is compensated by free energy change $\Delta\mathbf{G} = \mathbf{W} = \mathbf{E \bullet F \bullet n}$ and formation on metal (**Pt**) **electrode reduction potential E** .

$$\Sigma\mu_{Red} + \mathbf{E \bullet F \bullet n} = \Sigma\boldsymbol{\mu}_{Ox^{n+}} + \mathbf{n}\ \mu_{e^-} \qquad (6)$$

At **equilibrium** the **chemical potential** sum of initial compounds and products are equal and **reduced** form includes the compensating free energy change $\Delta\mathbf{G} = \mathbf{W} = \mathbf{E \bullet F \bullet n}$ forming on metal (**Pt**) **electrode reduction potential E** . Becomes obvious that **chemical potential** sum of **oxidized** form has the number **n** additional **chemical potential** of free electrons $\mathbf{n}\ \mu_{e^-}$ those values for all known **RedOx** systems are different and mostly laying in side interval between **-90 ÷ +90 kJ/mol**. Electrons $\mathbf{ne^-}$ are occupied metal (**Pt**) free electron gas solid phase and as pure solid compound has mol fraction concentration $\mathbf{X_{e^-} = 1}$. Expressing above mentioned meaning of **chemical potentials** (5) we can calculate the free energy change $\Delta\mathbf{G}$ in given **RedOx** system for **Red** form:

$$\Delta G^\circ_{Red} + \mathbf{R \bullet T \bullet ln}(X_{Red}) + \mathbf{E \bullet F \bullet n} = \Delta G^\circ_{Ox^{n+}} + \mathbf{R \bullet T \bullet ln}(X_{Ox^{n+}}) + \mathbf{n}\ \Delta G^\circ_{e^-} + \mathbf{n \bullet R \bullet T \bullet ln}(X_{e^-})$$
$$\Delta G^\circ_{Red} + \mathbf{R \bullet T \bullet ln}(X_{Red}) + \mathbf{E \bullet F \bullet n} = \Delta G^\circ_{Ox^{n+}} + \mathbf{R \bullet T \bullet ln}(X_{Ox^{n+}}) + \mathbf{n}\ \Delta G^\circ_{e^-} \qquad (7)$$

$$\Delta\mathbf{G} = \mathbf{E \bullet F \bullet n} = \Delta G^\circ_{Ox^{n+}} + \mathbf{n}\ \Delta G^\circ_{e^-} - \Delta G^\circ_{Red} + \mathbf{R \bullet T \bullet ln}\left(\frac{X_{Ox^{n+}}}{X_{Red}}\right)$$

$$\Delta\mathbf{G^\circ} = \mathbf{E^\circ \bullet F \bullet n} = \Delta G^\circ_{Ox^{n+}} + \mathbf{n}\ \Delta G^\circ_{e^-} - \Delta G^\circ_{Red} \qquad (8)$$

Standard free energy change **RedOx** system for **reduced** form **Red** is expressed as $\Delta G^\circ_{Red} = \mathbf{E^\circ \bullet F \bullet n}$ and for **oxidized** form $\mathbf{Ox^{n+}}$ as $\Delta G^\circ_{Ox^{n+}} = -\mathbf{E^\circ \bullet F \bullet n}$

$$\Delta G_{Red} = \mathbf{E \bullet F \bullet n} = \mathbf{E^\circ \bullet F \bullet n} + \mathbf{R \bullet T \bullet ln}\left(\frac{X_{Ox^{n+}}}{X_{Red}}\right);\ \Delta G_{Ox^{n+}} = -\mathbf{E \bullet F \bullet n} = -\mathbf{E^\circ \bullet F \bullet n} - \mathbf{R \bullet T \bullet ln}\left(\frac{X_{Ox^{n+}}}{X_{Red}}\right) \qquad (9)$$

With this equation we can calculate the **standard free-energy** change $\Delta\mathbf{G^\circ}$ for any **RedOx** system from the values of $\mathbf{E^\circ}$ in a table of r**eduction potentials** (Table 1) and the **free-energy** change $\Delta\mathbf{G}$ according (9) at known concentrations $\mathbf{X_{Red}}$ and $\mathbf{X_{Ox^{n+}}}$ of the each species form ($\Delta\mathbf{G_{Red}}$ and $\Delta\mathbf{G_{Ox^{n+}}}$) participating in the **RedOx** system.

Considerable **oxidation-reduction** reaction is composed from two **2RedOx** systems (half-reactions) using compounds reaction equivalence law $|\mathbf{+m' \bullet ne^-}| = |\mathbf{-n' \bullet me^-}|$ we have balanced **oxidation-redaction** reaction and can get the summary reaction of both half-reactions :

$$\mathbf{(-)\ Red_1 \Leftrightarrow Ox_1^{n+} + ne^-} \quad |\bullet \mathbf{m'}$$
$$\mathbf{(+)\ Ox_2^{m+} + me^- \Leftrightarrow Red_2} \quad |\bullet \mathbf{n'}$$

$\mathbf{m' \bullet Red_1 + n' \bullet Ox_2^{m+} \Rightarrow m' \bullet Ox_1^{n+} + n' \bullet Red_2}$ **; initial compounds** forming **products** direction of reaction.

With this equation we can calculate the **standard free-energy** change $\Delta\mathbf{G^\circ}$ for equi-molar amount of any **oxidation-reduction** reaction from the values of $\mathbf{E^\circ}$ in a table of r**eduction potentials** (Table 1)

$\Delta\mathbf{G^\circ} = \mathbf{m' \bullet} \Delta G^\circ_{Red1} - \mathbf{n' \bullet} \Delta G^\circ_{2Ox^{n+}} = \mathbf{m' \bullet} E^\circ_{Red1} \mathbf{\bullet F \bullet n} - \mathbf{n' \bullet} E^\circ_{2Ox^{n+}} \mathbf{\bullet F \bullet m} = (E^\circ_{Red1} - E^\circ_{2Ox^{n+}}) \mathbf{\bullet F \bullet (m'n = n'm)}$, where $\mathbf{n'm' = nm/N}$is equivalent - common number of electrons $\mathbf{e^-}$ involved in **RedOx** reaction can be less $\mathbf{n'm' \leq n \bullet m}$. by number **N** of common devider$\mathbf{Red_1}$ or $\mathbf{Ox_2^{m+}}$and the **free-energy** change $\Delta\mathbf{G}$ according (9) at known concentrations $\mathbf{X_{Red}}$ and $\mathbf{X_{Ox^{n+}}}$ of the each species ($\Delta\mathbf{G_{red}}$ and $\Delta\mathbf{G_{Ox^{n+}}}$) participating in the reaction.

$\Delta G = m' \bullet \Delta G_{Red1} - n' \bullet \Delta G_{2Ox^{n+}} = m' \bullet E_{Red1} \bullet F \bullet n - n' \bullet E_{2Ox^{n+}} \bullet F \bullet m = (E_{Red1} - E_{2Ox^{n+}}) \bullet F \bullet (m'n = n'm) =$

$= (E°_{Red1} - E°_{2Ox^{n+}}) \bullet F \bullet (m'n = n'm) + R \bullet T \bullet \ln\left(\frac{X^{m}_{1Ox^{n+}} \bullet X^{n}_{2Red}}{X^{m}_{1Red} \bullet X^{n}_{2Ox^{n+}}}\right)$, where(10)

$K_{eq} = \frac{X^{m}_{1Ox^{n+}} \bullet X^{n}_{2Red}}{X^{m}_{1Red} \bullet X^{n}_{2Ox^{n+}}}$ is homeostasis ratio as a multiple **products** over **initial** compounds concentrations.

Consider the reaction in which **acet-aldehyde** is **reduced** by the biological electron carrier **NADH**:

$H_3C\text{-}CH{=}O + NADH + H_3O^+ \Leftrightarrow H_3C\text{-}CH_2\text{-}OH + NAD^+ + H_2O$ (11)

acet-aldehyde ethanol

The relevant half-reactions and their E_{37} values are:

$RedNADH + H_2O \Leftrightarrow NAD^+ + H_3O^+ + 2e^-$ $E^1_{37} = -0.0590$ V (David Harris)

$OxCH_3CHO + 2H_3O^+ + 2e^- \Leftrightarrow CH_3CH_2OH + 2H_2O$ $E^2_{37} = 0.28169$ V (Suchotina)

By convention (10) balanced **n = 2 = m** number of electrons $2e^-\Delta E_{37}$ is expressed as E^1_{37} of the electron **donor** minus E^2_{37} of the electron **acceptor**. Because **acet-aldehyde** is **accepting** electrons from **NADH** in our example $\Delta E_{37} = E^1_{37} - E^2_{37} = -0.0590$ V $- 0.28169$ V $= -0.34069$ V, and **n** is **2.** Therefore

$\Delta G° = \Delta E_{37} \bullet F \bullet n = -0.34069$ V $\bullet$ 2 mol $\bullet$ 96485 C/mol $= -65.7429495$ kJ/mol

This is the free-energy change for the **oxidation-reduction** reaction at equilibrium state when $\Delta G = 0$ and so :

$\Delta G° = - R \bullet T \bullet \ln(K_{eq}); \frac{X_{NAD^+} \bullet X_{ethanol} \bullet X_{H_2O}}{X_{NADH} \bullet X_{Acetaldehyde} \bullet X_{H_3O^+}} = K_{eq} = e^{-\frac{\Delta G°}{R \bullet T}} = e^{-\frac{-65743}{8.314 \bullet 310.15}} = 1.18065 \bullet 10^{11}$

equilibrium is shifted far to **products** as shows Equilibrium constant **Keq = $1.181 \bullet 10^{11}$**. Anaerobic fermentation conditions NAD^+ concentration exceeds **NADH ratio 70** times at **pH = 7.36**. At presence of air oxygen O_2 **ratio** [NAD^+]/[**NADH**] is ten times higher **700** times over concentration **NADH**, what cause reaction condition to oxidize **ethanol** and **acet-aldehyde** as well known aerobic fermentation forms acetic acid. If **ethanol ratio** of concentrations is one as produced **ethanol** equal the same **acet-aldehyde** amount in aerobic fermentation: than calculated free energy change is positive $\Delta G = 5195$ **J/mol**produces 10% ethanol over acetaldehyde and reaction shifted toward acetic acid:

Anaerobic ΔG shifted to **ethanol** negative: $\Delta G = (E_{Red1} - E_{2Ox^{n+}}) \bullet F \bullet (m'n = n'm) + R \bullet T \bullet \ln\left(\frac{X^{m}_{1Ox^{n+}} \bullet X^{n}_{2Red}}{X^{m}_{1Red} \bullet X^{n}_{2Ox^{n+}}}\right) =$

$= (E°_{NADH} - E°_{Acetaldehyde}) \bullet F \bullet 2 + R \bullet T \bullet \ln\left(\frac{X_{NAD^+} \bullet X_{ethanol} \bullet X_{H_2O}}{X_{NADH} \bullet X_{Acetaldehyde} \bullet X_{H_3O^+}}\right)$

$= \Delta G° + R \bullet T \bullet \ln\left(\frac{X_{NAD^+} \bullet X_{ethanol} \bullet X_{H_2O}}{X_{NADH} \bullet X_{Acetaldehyde} \bullet X_{H_3O^+}}\right)$

$= -65.7429495 + R \bullet T \bullet \ln\left(\frac{X_{NAD^+} \bullet X_{ethanol} \bullet X_{H_2O}}{X_{NADH} \bullet X_{Acetaldehyde} \bullet X_{H_3O^+}}\right)$

$= -65.7429495 + 8.3144 \bullet 310.15 \bullet \ln(8.85352 \bullet 10^{10} \bullet 0.1)$

$= -65.7429495 + R \bullet T \bullet \ln\left(\frac{X_{NAD^+} \bullet X_{H_2O} \bullet X_{ethanol}}{X_{NADH} \bullet X_{H_3O^+} \bullet X_{Acetaldehyde}}\right) = \left(\frac{70}{1} \bullet \frac{55.21}{10^{-7.36}} \bullet \frac{1}{10}\right) =$ **-6680 J/mol**

Calculation the free-energy changes ΔG show possible modulation biological **RedOx reaction** at any concentrations **X** for the **reaction** driving forces and as regulation direction for favorable products formation.

http://aris.gusc.lv/BioThermodynamics/BioThermodynamics.pdf page 13 erythrocyte **-53.47kJ/mol** T=310,16 K

Cellular Oxidation of Glucose to Carbon Dioxide Requires Specialized Electron Carriers

The principles of **oxidation-reduction energetic** described above apply to the many metabolic reactions that involve electron e^- transfers. For example, in many organisms, the **oxidation** of **glucose** supplies **energy** for the production of **ATP**. The complete **oxidation** of **glucose**: $C_6H_{12}O_6 + 6O_{2aqua} + 6H_2O \Rightarrow 6HCO_3^- + 6H_3O^+ + \Delta G° + Q$

has a $\Delta G°$ of **-3049,55 kJ/mol**. This is a much larger release of free energy than is required for **ATP** synthesis erythrocyte and mitochondrial **pH = 7.36** use-**53.47kJ/mol** 55,4% of 100% **120** kJ/mol. Cells do not convert **glucose** to CO_{2aqua} in a single, high-**energy**-releasing reaction, but rather in a series of controlled reactions, some of which are **oxidations**. The **free energy** released in these **oxidation** steps is of the same order of magnitude as that required for **ATP** synthesis from **ADP**, with some **energy** to spare. Electrons e^- removed in these **oxidation** steps are transferred to **coenzymes** specialized for carrying two electrons $2e^-$, such as **NADH** and $FADH_2$(described below).

A Few Types of Coenzymes and Proteins Serve as Universal Electron Carriers

The multitude of **enzymes** that catalyze cellular **oxidation channel** electrons e^- from their hundreds **100** of different **substrates** into just a few types of universal **electron carriers**. The **reduction** of these **carriers** in catabolic processes results in the conservation of **free energy** released by **substrate oxidation**. NAD^+, $NADP^+$, **FMN**, and **FAD** are water-soluble **coenzymes** that undergo reversible ⇔**oxidation** and **reduction** in many of the electron-transfer e^- reactions of metabolism. The **nucleotides** NAD^+ and $NADP^+$ move readily from one **enzyme** to → another; the **flavin nucleotides FMN** and **FAD** are usually very tightly bound to the **enzymes**, called **flavo**-proteins, for which they serve as **prosthetic** groups. Lipid-soluble **quinones** such as **ubiquinone** and **plastoquinone** act as **electron carriers** and **proton donors** in the non-aqueous environment of membranes. **Iron-sulfur** proteins and **cytochromes**, which have tightly bound **prosthetic** groups that undergo reversible ⇔**oxidation** and **reduction**, also serve as electron e^- carriers in many **oxidation-reduction** reactions. Some of these proteins are water-soluble, but others are **peripheral** or **integral membrane** proteins.

We conclude this chapter by describing some chemical features of **nucleotide coenzymes** and some of the **enzymes** (**dehydrogenases** and **flavo**-proteins) that use them. The **oxidation- reduction** chemistry of **quinones**, **iron-sulfur** proteins, and **cytochromes** is discussed in Oxidative Phosphorylation and Photo-Phosphorylation.

NADH and NADPH Act with Dehydrogenases as Soluble Electron Carriers

Nicotin-amide adenine dinucleotide (NAD^+ in its **oxidized** form) and its close analog **nicotin-amide adenine dinucleotide phosphate** ($NADP^+$) are composed of two **2nucleotides** joined through their **phosphate** groups by a **phospho-anhydride** bond (Fig. 3). Because the **nicotinamide** ring resembles **pyridine**, these compounds are sometimes called **pyridine nucleotides**. The vitamin **niacin** is the source of the **nicotin-amide** moiety
In **nicotin-amide nucleotides**.

Both **coenzymes** undergo reversible ⇔**reduction** of the **nicotinamide ring** (Fig. 3).As a **substrate** molecule undergoes **oxidation** (**dehydrogenation**), giving up two **2** hydrogen **H** atoms, the **oxidized** form of the **nucleotide** (NAD^+ or $NADP^+$) **accepts** a **hydride** ion ($:H^-$ the equivalent of a proton H^+ and two **2** electrons e^-) and is transformed into the **reduced** form (**NADH** or **NADPH**). The second **2nd** proton H^+ removed from the **substrate** is released to the aqueous solvent H_2O. The half-reaction for each type of **nucleotide** is therefore

(**1**) **NADH** $+ H_2O \Leftrightarrow NAD^+ + H_3O^+ + 2e^-$ $E^1_{37} = -0.0590$ V (David Harris)

(**2**) **NADPH** $+ H_2O \Leftrightarrow NADP^+ + H_3O^+ + 2e^-$ $E^2_{37} = -0.0629$ V (CRC)

Reduction of NAD^+ or $NADP^+$ converts the **benzenoid ring** of the **nicotin-amide** moiety (with a fixed positive (+) charge on the ring **nitrogen N**) to the **quinonoid** form (with no charge ° on the **nitrogen N**). Note that the **reduced nucleotides** absorb light at **340 nm**: the **oxidized** forms do not (Fig. 13). The plus sign in the abbreviations NAD^+ and $NADP^+$ does not indicate the no charge on these molecules (they are both negatively (-) charged), but rather that the **nicotin-amide ring** is in its **oxidized** form, with a positive (+) charge on the **nitrogen** N^+ atom. In the abbreviations **NADH** and **NADPH**, the "**H**" denotes the added **hydride** ion. To refer to these **nucleotides** without specifying their **oxidation** state, we use **NAD** and **NADP**.

The total concentration of NAD^+**+ NADH** in most tissues is about 10^{-5}**M**; that of $NADP^+$ **+ NADPH** is about **10** times lower ↓. In many cells and tissues, the ratio of NAD^+(**oxidized**) to **NADH** (**reduced**) is high, favoring **hydride** H^- transfer from a **substrate** to NAD^+ to form **NADH**. By contrast, **NADPH** (**reduced**) is generally present in greater ↑ amounts than its **oxidized** form, $NADP^+$, favoring **hydride** H^- transfer from **NADPH** to a **substrate**. This reflects the specialized metabolic roles of the two **2coenzymes**: NAD^+ generally functions in **oxidations** - usually as part of a catabolic reaction; and **NADPH** is the usual coenzyme in **reductions** nearly always as part of anabolic reaction. A few **enzymes** can use either **coenzyme**. but most show a strong preference for one over the other. This functional specialization allows a cell to maintain two **2** distinct pools of **electron carrier**, swith two **2** distinct functions, in the same cellular compartment.

More than **200 enzymes** are known to catalyze reactions in which NAD^+ (or $NADP^+$) **accepts** a hydride $:H^-$ ion from a **reduced substrate** AH_2, or **NADPH** (or **NADH**) **donates** a hydride $:H^-$ ion to an **oxidized substrate A**. The general reactions are $H_3C\text{-}CH_2\text{-}OH + NAD^+ + H_2O \Leftrightarrow H_3C\text{-}CH{=}O + NADH + H_3O^+$(11)

Ethanol Acet-aldehyde

where **AH_2** is the **reduced substrate** and **A** the **oxidized substrate**. The general name for an **enzyme** of this type is **oxidoreductase;** they are also commonly called **dehydrogenases.** For example, **alcohol dehydrogenase** catalyzes the first **1st** step in the catabolism of **ethanol**, in which **ethanol** is **oxidized** to **acet-aldehyde**:

Red substrate $AH_2 + NAD^+ + H_2O \Leftrightarrow A + NADH + H_3O^+$(11)

Ox substrate $A + NADPH + H_3O^+ \Leftrightarrow AH_2 + NADP^+ + H_2O$(11)

Notice that one of the carbon atoms **$-CH_2-OH$** in **ethanol** has lost a hydrogen **H^-** atom as hydride and dissociates **$-OH \Rightarrow H^+$** proton ;the compound has been **oxidized** from an **alcohol** to an **aldehyde** (Fig. 3a).

When **NAD^+** or **$NADP^+$** is **reduced** the hydride **$:H^-$** ion could in principle the transferred to either side of the **nicotin-amide ring**: the front (**A** side) or the back (**B** side) as represented in Figure 3. Studies with isotopically labeled * **substrates** have shown that a given **enzyme** catalyzes either an **A**-type or **B**-type transfer, but not both. For example, yeast **alcohol dehydrogenase** and **lactate dehydrogenase** of vertebrate heart transfer a hydride **$:H^-$** ion to (or remove a hydride **$:H^-$** ion from) the **A** side of the **nicotin-amide ring**: they are classed as type **A dehydrogenases** to distinguish them from another group of **enzymes** that transfer a hydride **$:H^-$** ion to (or remove a hydride **$: H^-$** ion from) the **B** side of the **nicotin-amide ring** (Table 2).

The association between a **dehydrogenase** and **NAD** or **NADP** is relatively loose; the **coenzyme** readily diffuses from one **enzyme** to another, acting as a water-soluble **carrier** of electrons **e^-** from one **1**metabolite to another. For example, in the production of **alcohol** during **fermentation** of **glucose** by, yeast cells, a hydride **$:H^-$** ion is removed from **glycer-aldehyde 3-phosphate** by, one **1 enzyme** (**glycer-aldehyde 3-phosphate dehydrogenase**, a type **B enzyme**) and transferred to **NAD^+**. The **NADH** produced then leaves the **enzyme** surface and diffuses to another **enzyme** (**alcohol dehydrogenase**, a type **A enzyme**), which transfers a hydride **$:H^-$** ion to **acet-aldehyde**, producing **ethanol:** **Reduced** (calculated) At T=310 K (37° C) human glyceraldehyde3phosphate

Red $OHC-CHOH-CH_2OPO_3H^- + 2H_2O + HPO_4^{2-} \Leftrightarrow {}^{2-}O_3POOC-CHOHCH_2OPO_3H^- + 2e^- + 2H_3O^+$; $E^1 = -0.0273V$

(**Ox**) $NAD^+ + H_3O^+ + 2e^- \Leftrightarrow NADH + H_2O$ $E^2_{37} = -0.059$ **V** (David Harris) $\Delta G° = 6.117$ **kJ/mol**

glyceraldehyde3phosphate ⇔1,3-PhosphoGlycerate (**1**) $OHC-CHOH-CH_2OPO3H^- + NAD^+ + H_2O + HPO_4^{2-} => {}^{2-}O_3POOC-CHOHCH_2OPO_3H^- + NADH + H_3O^+$

Glyceraldehyde 3-phosphate =>1,3-phospho-glycerate (Carnegie Mellon Univ.)

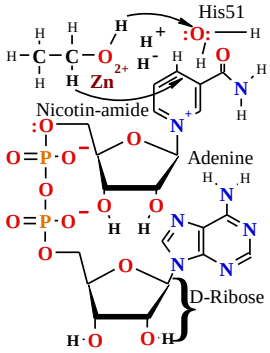
(**2**) $H_3C-CH=O + NADH + H_3O^+ \Leftrightarrow H_3C-CH_2-OH + NAD^+ + H_2O$ $\Delta G° = -55.498$ **kJ/mol**(calculated)

Sum$\Delta G° = 6.117$ **kJ/mol** + **-55.498 kJ/mol= -49.381 kJ/mol**

Notice that in the overall reaction there is no net production or consumption of **NAD^+** or **NADH**; the **coenzymes** function catalytically and are recycled repeatedly without a net change in the concentration **C** of **$[NAD^+]+[NADH]$.**

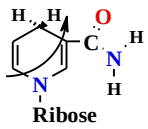
Figure 3.NAD and **NADP**$NAD^+ + H^- (2e^- + H^+) \Leftrightarrow NADH$**; Eo=-0.059V** standard potential T=310 K (37° C)

(**a**) **oxidized** **NAD^+** **$+2e^- + H_3O^+ \downarrow (H^- \downarrow ; + H_2O)$** **<=NADH+$H_2O$ reduced form product**



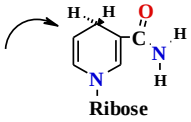
Hydrogen Transfer $H^- \downarrow$A

H_2O $+ H^- \downarrow$side A



B side or

↓ B side**↓** **$H^- + H_2O$**



(**a**) **Nicotin-amide adenine di-nucleotide** (**NAD^+**) and its **phosphorylated** analog **$NADP^+$** undergoes **reduction** to **NADH** and **NADPH**, **accepting** a hydride **$:H^-$ ion** (two electrons **$2e^-$** and one proton **H^+**) from an **oxidizable substrate**. The hydride **$:H^-$ ion** is added to either the front (the **A** side) or the back (the **B** side) of the planar **nicotin-amide ring** (seeTable2)

(**a**) **oxidized** **$NAD^+ + 2e^- + H_3O^+ \Leftrightarrow NADH + H_2O$reduced**

for **$NADP^+$** ribose **C**2'-**OH**hydr**oxyl** in **$NADP^+$** is esterified with **phosphate** $HO-PO_3^{2-}$ as ribose 2'**C-O-**PO_3^{2-}

↑A=log(Io/I) **Absorbance** measured **A=a•C•l** proportional to **NADH** concentration **C**into solution

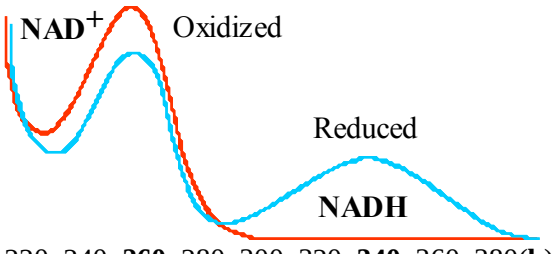


Figure 3. (**b**) The **UV** absorption spectra of **NAD^+** and **NADH**. **Reduction** of the **nicotin-amide ring** produces a new, broad absorption band with a maximum at **340 nm**. The production of **NADH** during an **enzyme-catalyzed** reaction can be conveniently followed by observing the appearance of the absorbance at **340 nm**; extinction coefficient **a= 6 200M^{-1}•cm^{-1}**molar absorbance **a=A/C/l** in Beer-Buger-Lambert's law **A=a•C•l** shows good sensitivity. **Table 2. Stereo specificity** of **Dehydrogenases** Wavelength (**nm**) ⟶That Employ **NAD^+** or **$NADP^+$** as **Coenzymes**

Enzyme **Coenzyme Stereo chemical** specificity **nicotin-amide ring** (**A** or **B**)

Iso-citrate dehydrogenase	**NAD^+**	**A**	
a-Keto-glutarate dehydrogenase	**NAD^+**		**B**
Glucose 6-phosphate dehydrogenase	**$NADP^+$**		**B**
Malate dehydrogenase	**NAD^+**	**A**	
Glutamate dehydrogenase	**NAD^+**or **$NADP^+$**	**B**	

Glyceraldehyde 3-phosphate dehydrogenase	**NAD^+**	**B**
Lactate dehydrogenase	**NAD^+**	**A**
Alcohol dehydrogenase	**NAD-**	**A**

Table 3. Some Enzymes (Flavo-proteins) That Employ Flavin Nucleotide Coenzymes

Enzyme	Flavin	Nucleotide Enzyme
Fatty acyl-CoA dehydrogenase	**FAD**	
Di-hydro-lipoyl dehydrogenase	**FAD**	Glycerol 3-phosphate dehydrogenase
Succinate dehydrogenase	**FAD**	Thio-redoxin reductase
NADH dehydrogenase Complex1	**FMN**	Glycolate dehydrogenase

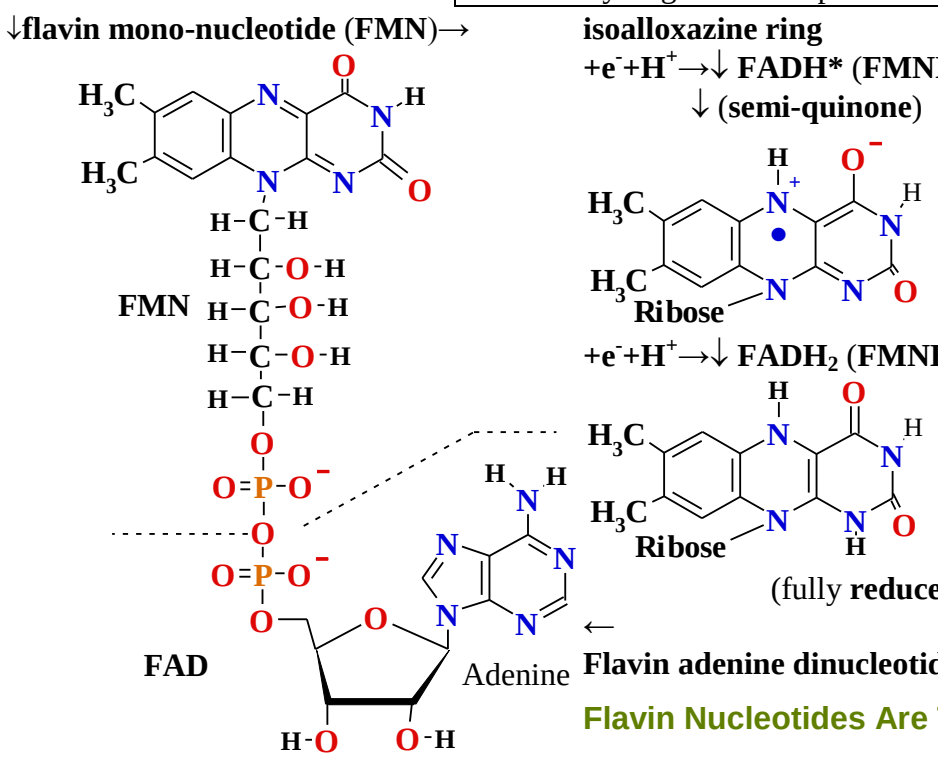


Figure 4. Structures of **oxidized** and **reduced FAD** and **FMN**. **FMN** consists of the structure above the dashed line shown on the **oxidized** (**FAD**) **structure**. The **flavin nucleotides accept** two hydrogen**2H** atoms (two electrons **2e⁻** and two protons **2H⁺**), both of which appear in the **flavin ring** system. When **FAD** or **FMN accepts** only one **1** hydrogen **H** atom, the **semi-quinone**, a stable free radical, forms.

Flavin Nucleotides Are Tightl y Bound in Flavo-proteins

Flavo-proteins (Table 3) are **enzymes** that catalyze **oxidation-reduction** reactions using either **flavin mono-nucleotide** (**FMN**) or **flavin adenine dinucleotide** (**FAD**) as **coenzyme** (Fig. 4). These **coenzymes** are derived from the vitamin **riboflavin**. The **fused ring** structure of **flavin nucleotides** (the **isoalloxazine ring**) undergoes reversible **reduction**, accepting either one **1** or two **2** electrons **e⁻** in the form of one **1** or two hydrogen**2H** atoms (each atom an electron **e⁻** plus a proton **H^+**) from a **reduced substrate**. The fully **reduced** forms are abbreviated **$FADH_2$** and **$FMNH_2$**. When a fully **oxidized flavin nucleotide accepts** only one **1** electron **e⁻** (one hydrogen **H** atom), the **semi-quinone** form of the **isoalloxazine ring** is produced, abbreviated **FADH*** and **FMNH*.** Because **flavo**-proteins can participate in either one-**1** or two electron **2e⁻** transfers, this class of proteins is involved in a greater diversity of reactions than the **pyridine nucleotide**-linked **dehydrogenases**.

Like the **nicotin-amide coenzymes** (Fig. 14-15), the **flavin nucleotides** undergo a shift in a major absorption band on reduction. **Oxidized flavo**-proteins generally have an absorption maximum near **570 nm**; when **reduced**, the absorption maximum shifts → to about **450 nm**. This change can be used to **assay** reactions involving a **flavo-protein**.

The **flavin nucleotide** in most **flavo**-proteins is bound rather tightly to the protein, and in some **enzymes**, such as **succinate dehydrogenase**, it is bound **covalently**. Such tightly bound **coenzymes** are properly called **prosthetic** groups. They do not transfer electrons **e⁻** by diffusing from one **1enzyme** to another second **2nd**; rather, they provide a means by which the **flavo**-protein can temporarily **hold** electrons **e⁻** while it **catalyzes** electron **e⁻** transfer from a **reduced substrate** to an electron **e⁻ acceptor**. One important feature of the **flavo**-proteins is the variability in the **standard reduction potential** (**E°**) of the bound **flavin nucleotide**. Tight association between the **enzyme** and **prosthetic** group confers on the **flavin ring** a **reduction potential E** typical of that particular **flavo-protein**, sometimes quite different from that of the **free flavin nucleotide**. **FAD** bound to **succinate dehydrogenase**, for example, has an **E_M = 0.0953 V** compared with **-0.3356 V** for **free FAD**. **Flavo**-proteins are often very complex: some have, in addition to a **flavin nucleotide**. tightly bound inorganic ions (iron **Fe^{n+}** or molybdenum **Mo^{n+}**, for example) capable of participating in electron **e⁻** transfer.

Summary

Living cells constantly perform work **W** and thus require energy **E** for the maintenance of highly organized. **structures**, for the **synthesis** or cellular components, for **movement**, for the generation of **electric currents**, for the production of **light**, and for many other processes. **Bio-energetic** is the quantitative study of energy **E** relationships and energy **E** conversions in biological systems. **Biological energy E** transformations obey the laws of **thermodynamics**. All chemical reactions are influenced by two **2** forces: the tendency to achieve the most stable bonding state (for which enthalpy, **H**, is a useful expression to show minimum reach at the most stable bonding state) and the tendency to achieve the highest ↑ degree of dispersed energy, **T•S** (called **bound energy**), which measure is the entropy, **S**. The production of entropy in a reaction as positive difference $\Delta S = S_{products} - S_{reactants} > 0$ is entropy increase ↑ from $S_{reactants}$ to $S_{products}$ due to dispersion of energy among the members of reaction. The net driving force in a reaction is the free-energy **G** decrease ↓ from $G_{reactants}$ to $G_{products}$ and negative difference $\Delta G = G_{products} - G_{reactants} < 0$, which represents the net effect of these two **2** factors: $T \bullet \Delta S + \Delta G = \Delta H$. Cells require sources of free energy **G** to perform work **W**.

The standard transformed free-energy change, $\Delta G°$, is a physical **constant** characteristic for a given **reaction** and can be calculated from the **equilibrium constant** K_{eq} for the reaction: $\Delta G° = -R \bullet T \bullet In(K_{eq})$. The actual free-energy change, ΔG, is a variable, which depends on, $\Delta G°$ and on the concentrations **C** of **reactants** and **products**: $\Delta G = \Delta G° + R \bullet T \bullet ln([products]/[reactants])$. When ΔG is large and negative $\Delta G < 0$, the reaction tends to go in the forward → direction; when it is large and positive $\Delta G > 0$, the reaction tends to go in the reverse ← direction; and when $\Delta G = 0$, the system is at **equilibrium**. The free-energy change ΔG for a reaction is independent of the **pathway** by which the reaction occurs only on **initial**($G_{reactants}$) and **final** ($G_{products}$)states. Free-energy changes ΔG are additive; the net chemical reaction that results from the successive occurrence of reactions sharing a common **intermediate** has an overall free-energy change $\Delta G_{reaction}$ that is the sum of the $\Delta G_{reaction} = \Delta G_{reaction1} + \Delta G_{reaction2}$ values for the individual reactions **reaction1** and **reaction2**.

ATP is the chemical link between catabolism and anabolism. It constitutes the energy currency of the living cell. Its **exoergonic** conversion to **ADP** and P_i, or to **AMP** and PP_i, is coupled to a large number of **endoergonic** reactions and processes. In general, it is not **ATP hydrolysis**, but the transfer of a **phosphoryl**, **pyro-phosphoryl**, or **adenylyl** group from **ATP** to a **substrate** or **enzyme** molecule that couples the energy of **ATP** breakdown to **endoergonic** transformations of **substrates**. By these group transfer reactions, **ATP** provides the energy for anabolic reactions, including the **synthesis** of informational molecules, and for the **transport** of molecules and ions across membranes against ⇔ concentration **C** gradients and electrical potential **E** gradients. Muscle contraction is one of several exceptions to this generalization; the conformational changes that produce muscle contraction are driven by **ATP** hydrolysis directly.

Cells contain other **metabolites** with large, negative $\Delta G < 0$, free energies of **hydrolysis**, including **phospho-enol-pyruvate**, **1,3-bis-phospho-glycerate**, and **phospho-creatine**. These high-energy compounds, like **ATP**, have a high **phosphoryl** group **transfer potential**; they are good **donors** of the **phosphoryl** group. **Thio-esters** also have high ↑ free energies **G** of **hydrolysis**.

Biological **oxidation-reduction** reactions can be described in terms of two **2**half-reactions (called **RedOx** systems), each with a characteristic **standard reduction potential, E°**. When two **2**electro-chemicalhalf-cells, each containing the components (**oxidized** and **reduced** forms) of a half-reaction, are connected, electrons e^- tend to flow → to the half-cell with the higher ↑**reduction potential E**. The strength of this tendency is proportional to the difference between the two **2reduction potentials** (ΔE) and is a function of the concentrations **C** of **oxidized** [**Ox**] and **reduced** [**Red**] species. The standard free-energy change $\Delta G°$ for an **oxidation-reduction** reaction is directly proportional to the difference in **standard reduction potentials** $\Delta E°$ of the two **2**half-cells: $\Delta G° = F \bullet n \bullet \Delta E°$.

Many biological **oxidation** reactions are **dehydrogenation** in which one **1** or two **2** hydrogen **H** atoms (electron e^- and proton H^+) are transferred from a **substrate** to a hydrogen **H acceptor**. **Oxidation-reduction** reactions in cells involve specialized electron **e^-carriers**. **NAD** and **NADP** are the freely diffusible **coenzymes** of many **dehydrogenases**. Both NAD^+ and $NADP^+$ **accept** two **2** electrons e^- and one **1** proton H^+. **FAD** and **FMN**, the **flavin nucleotides**, serve as tightly bound **prosthetic** groups of **flavo**-proteins. They can **accept** either one **1** or two **2** electrons e^-. In many organisms, a central energy-conserving process is the **stepwise oxidation** of **glucose** to CO_2, in which some of the energy of **oxidation** is conserved in **ATP** as electrons e^- are passed to O_2.

Further Reading

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Biological Oxidation- Reduction Reactions

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Problems1. Entropy Changes during Egg Development

Consider a system consisting of an egg in an incubator. The white and yolk of the egg contain proteins, **carbohydrates**, and **lipids**. If fertilized, the egg is transformed from a single meiotic cell to a complex mitotic cells in organism. Discuss this **irreversible** process in terms of the entropy changes Δ**S** in the **system**, **surroundings**, and **universe**. Be sure that you first clearly define the **system** and **surroundings-environment**.

2. Calculation of $\Delta G°$ from Equilibrium Constants

Calculate the standard free-energy changes Δ**G°** the following metabolically important **enzyme-catalyzed** reactions at **25°C** and **pH 7.0** from the equilibrium constants $\mathbf{K_{eq}}$ given.

(**a**) $\Delta G° = - R•T•\ln(K_{eq}) =$ = **-4.752 kJ/mol**

Glutamate + oxalo-acetate⇔aspartate amino-transferase⇔ aspartate + α-keto-glutarate $\mathbf{K_{eq}}$**= 6.8**

(**b**) $\Delta G° = - R•T•\ln(K_{eq}) =$ = **7.553 kJ/mol**

Di-hydroxy-acetone phosphate⇔triose phosphate isomerase⇔ glyceraldehyde 3-phosphate $\mathbf{K_{eq}}$**= 0.0475**

(**c**) $\Delta G° = - R•T•\ln(K_{eq}) =$ = **-13.727 kJ/mol**

Fructose 6-phosphate^{-} + ATP^{4-}⇔phospho-fructo-kinase⇔fructose 1,6-bisphosphate^{2-} + ADP^{3-} $\mathbf{K_{eq}}$**= 254**

$\Delta G° = - R•T•\ln(K_{eq})$; $\Delta G = \Delta G° + R•T•\ln(K_{eq})$

3. Calculation of Equilibrium Constants K_{eq} from $\Delta G°$

Calculate the equilibrium constants $\mathbf{K_{eq}}$for each of the following reactions at **pH 7.0** and **25°C**, using the Δ**G°** values of Table 1-1 $\mathbf{K_{eq} = EXP}\left(\frac{\Delta G^{o}}{R \bullet T}\right)$ corrected by $\mathbf{\Delta G° = \Delta G°_{o} + G°_{HPO4} + G°_{H3O+} - G°_{H2PO4} - G°_{H2O}}$=

= -13.8 + (-1282) + (-284.7) - (-1323) - (-306.7) = 49.306 kJ/mol

$\mathbf{K°_{7eq}=3}$; $\frac{[Glc] \bullet [HPO_4^{2-}] \bullet [H_3O^+]}{[Glc-6P^-] \bullet [H_2O]^2} = \mathbf{3•10^{-7} = K°_{H2PO4} • K_{eq} = 1.15•10^{-9} • 261.573 = K°_{eq}; \Delta G°_{eq} = 37.2\ kJ/mol}$

$H_2PO_4^- + H_2O \Leftrightarrow HPO_4^{2-} + H_3O^+$; $\mathbf{K°_{H2PO4}} = \mathbf{1.1469•10^{-9}}$ (Kortly Shucha)

(**a**) Glucose 6-phosphate^{-}+H_2O⇔glucose6-phosphatase⇔glucose+$H_2PO_4^-$;$\mathbf{K_{eq}=261.573; \Delta G°_{o}=-13.8\ kJ/mol}$

Glucose 6-phosphate^{-}+2H_2O⇔glucose6-phosphatase⇔glucose+HPO_4^{2-}+H_3O^+;$\mathbf{K°_{eq}=3•10^{-7}; \Delta G° = 49.3\ kJ/mol}$

Equilibrium is shifted toward formation of Glucose 6-phosphate^{-} and is affected by $\mathbf{H_3O^+}$ concentration.

(**b**) $\mathbf{K°_{eq}} = \frac{[Glc] \bullet [Gal]}{[Lactose] \bullet [H_2O]} =$ **610.221 ; $\Delta G°$ = -15.9 kJ/mol**

(**b**) Lactose + H_2O ⇔β-galactosidase⇔ glucose + galactose $\mathbf{K_{eqH2O}}$ **= 33773**

(**c**) Malate⇔fumarase⇔fumarate + H_2O $\mathbf{K_{eqH2O}}$ **= 0.004615**

; $\mathbf{K°_{eq}} = \frac{[Fumarate] \bullet [H_2O]}{[Malate]} =$ **0.28636 ; $\Delta G°$ = 3.1 kJ/mol4. Experimental Determination of $K°_{eq}$ and $\Delta G°$**

If a **0.1 M** solution of **glucose 1-phosphate** is incubated with a catalytic amount of **phospho-gluco-mutase**, the **glucose 1-phosphate** is transformed to **glucose 6-phosphate**. At equilibrium, the concentrations of the reaction components are:

Glucose 1-phosphate⇔phospho-gluco-mutase⇔glucose 6-phosphate^{-}

$\mathbf{[Glc1P^-] = 4.5•10^{-3}\ M}$ $\mathbf{9.6•10^{-2}\ M = [Glc6P^-]}$

Calculate $\mathbf{K°_{eq}=[Glc6P^-]/[Glc1P^-]=21.3}$ and $\mathbf{\Delta G°=-R•T•\ln(21.33)=-7.586\ kJ/mol}$ for this reaction at **25°C.**

5. Experimental Determination of $\Delta G°$ for ATP Hydrolysis

A direct measurement of the standard free-energy change Δ**G°** associated with the **hydrolysis** of **ATP** is technically demanding because the minute amount of **ATP** remaining at equilibrium is difficult to measure accurately. The value of Δ**G°** can be calculated indirectly, however, from the equilibrium constants of two **2** other **enzymatic** reactions having less favorable equilibrium constants:

$\mathbf{\Delta G°_1 = \Delta G°_{o} + G°_{HPO4} + G°_{H3O+} - G°_{H2PO4} - G°_{H2O} = 49.306\ kJ/mol}$

$H_2PO_4^- + H_2O \Leftrightarrow HPO_4^{2-} + H_3O^+$; $\mathbf{K°_{H2PO4}} = \mathbf{1.1469•10^{-9}}$ (KortlyShucha)

Glucose-6-phosphate^{-} + H_2O => glucose + $H_2PO_4^-$; $\mathbf{K_{eq}= 270\ ;}$ $\mathbf{\Delta G°_{eq} = -13.879\ kJ/mol}$

Glucose 6-phosphate^{-} + H_2O⇔glucose + $H_2PO_4^-$; $\mathbf{K°_{o}}$ **= 261.573 ;** $\mathbf{\Delta G°_{o} = -13.8\ kJ/mol}$

$\mathbf{K°_{eq} \bullet K°_{H2PO4} = K°_{eq1}} = \frac{[Glc] \bullet [HPO_4^{2-}] \bullet [H_3O^+]}{[Glc - 6P^-] \bullet [H_2O]^2} = \mathbf{3.1 \bullet 10^{-7}};$

$\mathbf{\Delta G°_{eq1} = 37.16\ kJ/mol}$

(**1**) Glucose-6-phosphate$^-$ + 2 H_2O => glucose + HPO_4^{2-} + H_3O^+ ; $\mathbf{K°_{eq1} = 3.097 \bullet 10^{-7}; \Delta G°_1 = 49.3\ kJ/mol}$

(**2**) ATP^{4-} + glucose => ADP^{3-} + glucose 6-phosphate$^-$; $\mathbf{K_{eq2} = 890}$

$\mathbf{K°_{eq2}} = \frac{[ADP^{3-}] \bullet [Glc - 6P^-]}{[Glc] \bullet [ATP^{4-}]} = \mathbf{890\ ; \Delta G°_{eq2} = -16.836\ kJ/mol}$

Using this information, calculate the standard free energy $\Delta\mathbf{G°}$ of hydrolysis of **ATP** at **25°C**.

$\mathbf{K°_{eq3}} = \frac{[ADP^{3-}] \bullet [HPO_4^{2-}] \bullet [H_3O^+]}{[ATP^{4-}] \bullet [H_2O]^2} = \mathbf{K°_{eq1} \bullet K°_{eq2}} = \frac{[Glc] \bullet [HPO_4^{2-}] \bullet [H_3O^+]}{[Glc6P^-] \bullet [H_2O]^2} \bullet \frac{[ADP^{3-}] \bullet [Glc6P^-]}{[Glc] \bullet [ATP^{4-}]}$

$\mathbf{\Delta G°_3 = \Delta G°_o + G°_{HPO4} + G°_{H3O+} - G°_{H2PO4} - G°_{H2O} = 32.606\ kJ/mol}$

(**3**)$ATP^{4-} + 2H_2O => ADP^{3-} + HPO_4^{2-} + H_3O^+$; $\mathbf{K°_{eq3} = 0.0002756; \Delta G°_{eq3} = 20.32\ kJ/mol; \Delta G°_{123} = 32.464\ kJ/mol}$

$\mathbf{K_{eq}°_o} = \frac{[ADP^{3-}] \bullet [H_2PO_4^-]}{[ATP^{4-}] \bullet [H_2O]^2} = \mathbf{K°_{eq3}/K°_{H2PO4} = 240300}$; $\mathbf{\Delta G_{eq}°_o = -30.714\ kJ/mol}$

(**3**) $ATP^{4-} + H_2O => ADP^{3-} + H_2PO_4^{2-}$; $\mathbf{K°_o = 220409\ ; \Delta G°_o = -30.500\ kJ/mol}$

6. Difference between $\Delta\mathbf{G°}$ and $\Delta\mathbf{G}$ Consider the following inter conversion, which occurs in **glycolysis** :
Fructose 6-phosphate$^-$ ⇔ glucose 6-phosphate$^-$; $\mathbf{K°_{eq} = 1.97}$

$\mathbf{K°_{eq}} = \frac{[Glc6P^-]}{[Fruc6P^-]} = \mathbf{1.97}$; $\mathbf{\Delta G° = -R \bullet T \bullet \ln(K°_{eq}) = -1.5399\ kJ/mol}$

(**a**) What is $\Delta\mathbf{G°}$ for the reaction (assuming that the temperature is **25°C**)?

(**b**) If the concentration of [Fruc6P$^-$] is adjusted to **1.5 M** and that of

[Glc6P$^-$] is adjusted to **0.5 M**, what is $\Delta\mathbf{G}$?

(**c**) Why are $\Delta\mathbf{G°}$ and $\Delta\mathbf{G}$ different? $\Delta\mathbf{G} = \Delta\mathbf{G°} + \mathbf{R \bullet T \bullet \ln}(\frac{[Glc6P^-]}{[Fruc6P^-]}) = \mathbf{-4.263.4\ kJ/mol}$

7. Dependence of $\Delta\mathbf{G}$ on pH. The free energy $\Delta\mathbf{G}$ released by the **hydrolysis** of **ATP** under standard conditions at **pH=7** is $\mathbf{\Delta G°_o = -30.5\ kJ/mol}$. If **ATP** is **hydrolyzed** under standard conditions but at **pH=5.0**, is more or less free energy released? Why?

$\mathbf{K°_{eq3}} = \frac{[ADP^{3-}] \bullet [HPO_4^{2-}] \bullet [H_3O^+]}{[ATP^{4-}] \bullet [H_2O]^2} = \mathbf{1.94 \bullet 10^{-6}; K°_{eq3}/K°_{H2PO4} = K°_o} = \frac{[ADP^{3-}] \bullet [H_2PO_4^-]}{[ATP^{4-}] \bullet [H_2O]^2} = \mathbf{220409}$

$\mathbf{\Delta G°_3 = \Delta G°_o + G°_{HPO4} + G°_{H3O+} - G°_{H2PO4} - G°_{H2O} = 32.606\ kJ/mol}$

$\Delta\mathbf{G} = \Delta\mathbf{G°} + \mathbf{R \bullet T \bullet \ln} \frac{[ADP^{3-}] \bullet [HPO_4^{2-}] \bullet [H_3O^+]}{[ATP^{4-}] \bullet [H_2O]^2} = \mathbf{-10.11\ kJ/mol}$ (**pH=0**); at **T=298 K** (**25 °C**)

-38.65 kJ/mol (**pH=5**); **-50.06 kJ/mol** (**pH=7**); **-52.12 kJ/mol** (**pH=7.36**); **-57.88 kJ/mol** (**pH=8.37**)
-11.83 kJ/mol (**pH=0**); at **T = 310 K** (**37 °C**) in mitochondria
-41.52 kJ/mol (**pH=5**); **-53.39 kJ/mol** (**pH=7**); **-55.53 kJ/mol** (**pH=7.36**); **-61.53 kJ/mol** (**pH=8.37**)

8. The $\Delta\mathbf{G°}$ for Coupled Reactions

Glccose 1-phosphate$^-$ is converted into fructose 6-phosphate$^-$ in two **2** successive reactions:
Glucose 1-phosphate$^-$=> glucose 6-phosphate$^-$; $\mathbf{\Delta G°_1 = -7.3\ kJ/mol}$

Glucose 6-phosphate$^-$=>.fructose 6-phosphate$^-$ $\mathbf{\Delta G°_2 = +1.7\ kJ/mol}$

Using the $\Delta\mathbf{G°}$ values in Table 1.1, calculate the equilibrium constant,

$\mathbf{\Delta G° = \Delta G°_1 + \Delta G°_2 = -7.3 + 1.7 = -5.6\ kJ/mol}$ for the sum of the two **2** reactions at **25°C**:

Glucose 1-phosphate$^-$=>fructose 6-phosphate$^-$; $\mathbf{K°_{eq} = K_{eq1} \bullet K_{eq2} = 9.57309}$

9. Strategy for Overcoming an Unfavorable Reaction: ATP-Dependent Chemical Coupling The **phosphorylation** of **glucose** to glucose 6-phosphate$^-$ is the initial step in the catabolism of **glucose**. The direct **phosphorylation** of **glucose** by $\mathbf{H_2PO_4^-}$ and $\mathbf{HPO_4^{2-}}$ is described by the equation at **T = 310.16 K**:

(**a**)Glucose + $\mathbf{H_2PO_4^-}$=>. glucose 6-phosphate$^-$ + H_2O, $\Delta\mathbf{G_o°}$ **=13.8 kJ/mol**

$$\mathbf{K°_a}=\frac{[Glc6P^-]\bullet[H_2O]}{[Glc]\bullet[H_2PO_4^-]};\ \mathbf{K°_{a2}\bullet K°_{H2PO4}}=\frac{[Glc6P^-]\bullet[H_2O]^2}{[Glc]\bullet[HPO_4^{2-}]\bullet[H_3O^+]}\bullet\frac{[HPO_4^{2-}]\bullet[H_3O^+]}{[H_2PO_4^-]\bullet[H_2O]}$$

(**a2**) Glucose + $\mathbf{HPO_4^{2-}}$ + H_3O^+=>.glucose 6-phosphate$^-$ + 2 H_2O, $\Delta\mathbf{G°}$ **= -49.306 kJ/mol**

$$\mathbf{K°_{a2}}=\frac{[Glc6P^-]\bullet[H_2O]^2}{[Glc]\bullet[HPO_4^{2-}]\bullet[H_3O^+]}=\frac{[Glc6P^-]\bullet\left(1+\frac{[H_3O^+]}{K_{H2PO4}\bullet[H_2O]}\right)\bullet[H_2O]^2}{[Glc]\bullet 4.8\bullet[H_3O^+]}=\mathbf{2.0119\bullet10^{+8}}$$

$$\mathbf{K°_{H2PO4}}=\frac{[HPO_4^{2-}]\bullet[H_3O^+]}{[H_2PO_4^-]\bullet[H_2O]};\ \mathbf{P_i = 4.8\ mM = [H_2PO_4^-]+[HPO_4^{2-}]};\ \mathbf{[HPO_4^{2-}] = 4.8 -}\frac{[HPO_4^{2-}]\bullet[H_3O^+]}{K_{H2PO4}\bullet[H_2O]}=$$

$$\mathbf{[HPO_4^{2-}]+[HPO_4^{2-}]}\frac{[H_3O^+]}{K_{H2PO4}\bullet[H_2O]}=\mathbf{4.8 = [HPO_4^{2-}]\bullet\left(1+\frac{[H_3O^+]}{K_{H2PO4}\bullet[H_2O]}\right);}$$

$$\mathbf{[HPO_4^{2-}]}=\frac{4.8}{\left(1+\frac{[H_3O^+]}{K_{H2PO4}\bullet[H_2O]}\right)};\ \mathbf{[Glc6P^-]}=\frac{2.012\bullet[Glc]\bullet4.8\bullet[H_3O^+]}{\left(1+\frac{[H_3O^+]}{K_{H2PO4}\bullet[H_2O]}\right)\bullet[H_2O]^2}=\mathbf{1.0574\bullet10^{-5}M}$$

(**a**) Calculate the equilibrium constant $\mathbf{K°_a}$ for the above reaction. In the rat **hepatocyte pH=7.36** and at **pH=7** the physiological concentrations of **glucose** and $[\mathbf{H_2PO_4^-}]+[\mathbf{HPO_4^{2-}}]$ are maintained at approximately **4.8 mM**. What is the equilibrium concentration of glucose 6-phosphate$^-$ obtained by the direct **phosphorylation** of **glucose** by $\mathbf{H_2PO_4^-}$ + $\mathbf{HPO_4^{2-}}$? Respectively [**Glc6P**$^-$] = $\mathbf{8.5\bullet10^{-8}M}$ and $\mathbf{1.275\bullet10^{-7}M}$ (**pH 7.36** and **7**)
Does this reaction represent a reasonable metabolic step for the catabolism of **glucose**? Explain.

(**b**) In principle, at least, one way to increase the concentration of glucose 6-phosphate$^-$ is to drive the equilibrium reaction to the right by increasing the intracellular concentrations of **glucose** and $\mathbf{H_2PO_4^-}$ + $\mathbf{HPO_4^{2}}$. Assuming a fixed concentration of $\mathbf{H_2PO_4^-}$ + $\mathbf{HPO_4^{2-}}$ at **4.8 mM**, how high would the intracellular concentration of **glucose** have to be to give an equilibrium concentration of glucose 6-phosphate$^-$ of [**Glc6P**$^-$]=**250 µM** (normal physiological concentration)'? Would this route be physiologically reasonable, given that the maximum solubility of **glucose** is less than **1 M**?

$$\mathbf{[Glc]}=\frac{[Glc6P^-]\bullet\left(1+\frac{[H_3O^+]}{K_{H2PO4}\bullet[H_2O]}\right)\bullet[H_2O]}{K_{a2}\bullet4.8/1000\bullet[H_3O^+]}=\mathbf{23.64M}\ \text{at}\ \mathbf{pH = 7.36}$$

(**c**) The **phosphorylation** of **glucose** in the cell is coupled to the **hydrolysis** of **ATP**; that is, part of the free energy of **ATP hydrolysis** is utilized to effect the **endoergonic phosphorylation** of **glucose** at **T = 310.16 K**:

(**1**) Glucose + $\mathbf{H_2PO_4^-}$ ⇔. glucose 6-phosphate$^-$ + H_2O **;ΔG°$_{o1}$ =13.8 kJ/mol**
(**2**)ATP^{4-} + H_2O ⇔ ADP^{3-} + $H_2PO_4^-$; **K°$_o$= 220409 ;** Δ**G°$_{o2}$= -30.500 kJ/mol**
Sum: ATP^{4-} + glucose ⇔ ADP^{3-} + glucose 6-phosphate$^-$; **ΔG°$_o$ = -16.7 kJ/mol** at $\mathbf{T_o}$ **= 298.16 K**

$$\mathbf{K°_o}=\mathbf{842.63}\ \text{<=}\ \mathbf{EXP(-\Delta G°/R/T)} = \underline{\mathbf{649.3 = K°}};\ \mathbf{K°_{eqo}}=\frac{[ADP^{3-}]\bullet[Glc6P^-]}{[ATP^{4-}]\bullet[Glc]}=\mathbf{890\ ;\ \Delta G°_{eqo}= -16.836\ kJ/mol}$$

(**1**) glucose + HPO_4^{2-} + H_3O^+⇔ Glucose-6-phosphate$^-$ + 2 H_2O **;ΔG°$_1$= -49.3 kJ/mol**
(**2**)ATP^{4-}+2H_2O⇔ADP^{3-}+HPO_4^{2-}+H_3O^+ **; ΔG°$_2$= 32.606 kJ/mol**
Sum: ATP^{4-} + glucose ⇔ ADP^{3-} + glucose 6-phosphate$^-$ **;ΔG°= -16.694 kJ/mol**

$\Delta G^{\circ}_2 = \Delta G^{\circ}_{o2} + G^{\circ}_{HPO4} + G^{\circ}_{H3O+} - G^{\circ}_{H2PO4} - G^{\circ}_{H2O} = 32.606$ **kJ/mol**

$$[\mathbf{Glc}] = \frac{[ADP^{3-}] \bullet [Glc6P^{-}]}{K^{o} \bullet [ATP^{4-}]} = \frac{1.32 \bullet 0.25/1000}{649.2998 \bullet 3.38} = \mathbf{1.504 \bullet 10^{-7} M}$$ not depend on concentration [H_3O^+]

Calculate **K** for the overall reaction. For the **ATP**-dependent **phosphorylation** of **glucose**, what concentration of glucose is needed to achieve a **250 μM** intracellular concentration of glucose 6-phosphate when the concentrations of **ATP** and **ADP** are **3.38** and **1.32 mM**, respectively? Does this coupling process provide a feasible route, at least in principle, for the **phosphorylation** of **glucose** in the cell? Explain.

(**d**) Although coupling **ATP hydrolysis** to **glucose phosphorylation** makes thermodynamic sense, how this coupling is to take place has not been specified. Given that coupling requires a common intermediate, one conceivable route is to use **ATP hydrolysis** to raise the intracellular concentration of $H_2PO_4^-$ + HPO_4^{2-} and thus drive the unfavorable phosphorylation of glucose by $H_2PO_4^-$ + HPO_4^{2-}. Is this ~i reasonable route? (Think about the solubility products of metabolic intermediates.)

(**e**) The **ATP**-coupled **phosphorylation** of **glucose** is catalyzed in **hepatocytes** by the enzyme **gluco kinase**. This enzyme binds **ATP** and **glucose** to form a **glucose-ATP**-enzyme complex, and the **phosphoryl** group is transferred directly from **ATP** to **glucose**. Explain the advantages of this route.

10. Calculations of ΔG° for ATP-Coupled Reactions From data in Table 1-2 calculate the **ΔG°** value for the reactions:

(**1**$_o$) Phospho Creatine$^-$ + H_2O⇔Creatine + $H_2PO_4^-$	**;ΔG°_{1o}= -43.095 kJ/mol**
$\Delta G^{\circ}_{1`} = \Delta G^{\circ}_{1o} + G^{\circ}_{HPO4} + G^{\circ}_{H3O+} - G^{\circ}_{H2PO4} - G^{\circ}_{H2O} = 20.011$ **kJ/mol**	
(**1**) Phospho Creatine$^-$ + 2 H_2O⇔Creatine + HPO_4^{2-} + H_3O^+	**;ΔG°_1= 20.011 kJ/mol**
(**2**)ADP^{3-}+HPO_4^{2-}+H_3O^+⇔ ATP^{4-}+2H_2O	**; ΔG°_2= -32.606 kJ/mol**
(**a**) Phosphocreatine$^-$ + ADP^{3-}⇔creatine + ATP^{4-}	**;ΔG°_a= -12.595 kJ/mol**
(**1$_{bo}$**) fructose + $H_2PO_4^-$⇔ fructose 6-phosphate$^-$ + H_2O	**;ΔG°_{1bo}= -15.899 kJ/mol**
$\Delta G^{\circ}_{1b`} = \Delta G^{\circ}_{1bo} + G^{\circ}_{HPO4} + G^{\circ}_{H3O+} - G^{\circ}_{H2PO4} - G^{\circ}_{H2O} = -79.005$ **kJ/mol**	
(**1**) fructose 6-phosphate$^-$ + 2 H_2O⇔ fructose + HPO_4^{2-} + H_3O^+	**;ΔG°_{1b}= -79.005 kJ/mol**
(**2**)ADP^{3-}+HPO_4^{2-}+H_3O^+⇔ ATP^{4-}+2H_2O	**; ΔG°_{2b}= -32.606 kJ/mol**
(**b**) ATP^{4-}+ fructose ⇔ ADP^{3-} + fructose 6-phosphate$^-$	**;ΔG°_b= -111.6 kJ/mol**

11. Coupling ATP Cleavage to an Unfavorable Reaction.
To explore the consequences of coupling **ATP hydrolysis** under physiological conditions to a thermodynamically unfavorable biochemical reaction, consider the hypothetical transformation **X**□**Y**, for which **ΔG° 20 kJ/mol**.

(**a**) What is the ratio [**Y**]/[**X**I at equilibrium? $\mathbf{K^{\circ}_o = 3.135 \bullet 10^{-4}}$

(**b**) Suppose **X** and **Y** participate in a sequence of reactions during which ATP^{4-} is **hydrolyzed** to ADP^{3-} and $H_2PO_4^-$, The overall reaction is :

(**1**) **X** ⇔**Y**	; ΔG°_{o1} = 20 kJ/mol
(**2**)ATP^{4-} + H_2O ⇔ ADP^{3-} + $H_2PO_4^-$; **K°_o= 220409**	**; ΔG°_{o2}= -30.500 kJ/mol**
X + ATP^{4-} + H_2O ⇔**Y** + ADP^{3-} + $H_2PO_4^-$; $\Delta G^{\circ}_o = \Delta G^{\circ}_{o1} + G^{\circ}_{o2} = -10.500$ **kJ/mol**	

$$\mathbf{K^{\circ}_{eqo}} = \frac{[ADP^{3-}] \bullet [H_2PO_4^-] \bullet [Y]}{[ATP^{4-}] \bullet [H_2O] \bullet [X]} = \mathbf{69.1} \; ; \mathbf{K^{\circ}_{eqo}} \bullet [H_2O] = \frac{[Y]}{[X]} = \mathbf{3810}$$

$$\frac{[Y]}{[X]} = \frac{[ATP^{4-}]\bullet[H_2O]\bullet K^{o}_{eqo}\bullet\left(1+\frac{K_{H2PO4}\bullet[H_2O]}{[H_3O^+]}\right)}{8.05/1000\bullet[ADP^{3-}]} = \mathbf{1.994\bullet10^{6}}\ ;[\mathbf{H_2PO_4^-}] = \frac{8.05/1000}{1+\frac{K_{H2PO4}\bullet[H_2O]}{[H_3O^+]}}$$

$\mathbf{P_i = 8.05\ mM = [H_2PO_4^-]+[HPO_4^{2-}]}$; $\mathbf{[H_2PO_4^-] = 8.05/1000 -} \frac{[H_2PO_4^-]K_{H2PO4}\bullet[H_2O]}{[H_3O^+]}$;

Calculate [**Y**]/[**X**] for this reaction at equilibrium. Assume that the concentrations of [$\mathbf{ATP^{4-}}$], [$\mathbf{ADP^{3-}}$], and ([$\mathbf{H_2PO_4^-}$]+ [$\mathbf{HPO_4^{2-}}$])are all **1 M** when the reaction is at equilibrium **T = 310.16 K**.

(**c**) We know that [ATP^{4-}], [ADP^{3-}], and [$\mathbf{H_2PO_4^-}$]l are not **1 M** under physiological conditions. Calculate [**Y**]/[**X**] for the **ATP-coupled** reaction when the values of [ATP^{4-}], [ADP^{3-}], and [$\mathbf{H_2PO_4^-}$]l are those found in rat **myocytes** (Table 1-3).

12. Calculations of ΔG at Physiological Concentrations.

Calculate the physiological **ΔG** (not **ΔG°**) for the reaction :at**T=310.16 K**

Phospho creatine$^-$ + ADP^{3-} ⇔ creatine + ATP^{4-} **;ΔG°= -12.5 kJ/mol**

$\mathbf{\Delta G=\Delta G^{\circ}+R\bullet T\bullet ln}\frac{[ATP^{4-}]\bullet[Cr]}{[ADP^{3-}]\bullet[PCr^-]}$ **=- -13.215 kJ/mol**

at **37 °C** as it occurs in the **cytosol** of neurons, in which **phospho creatine**$^-$ is present at [$\mathbf{PCr^-}$] **= 4.7 mM**, creatine at [**Cr**] **= 1.0 mM**, ADP^{3-} at **0.73 mM**, and ATP^{4-} at **2.6 mM**.

13. Free Energy Required for ATP Synthesis under Physiological Conditions.

In the **cytosol** of rat **hepatocytes**, the **mass-action ratio** is :

$\mathbf{R^{\circ}_o}=\frac{[ATP^{4-}]}{[ADP^{3-}]\bullet([HPO_4^{2-}]+[H_2PO_4^-])}$ **=5.33•10^{-2} M^{-1}** ; at **37 °C T = 310.16 K**

$\mathbf{P_i}$ = [$\mathbf{H_2PO_4^-}$]+[$\mathbf{HPO_4^{2-}}$] ; $\mathbf{[H_2PO_4^-]} = \frac{[ATP^{4-}]}{[ADP^{3-}]\bullet R^{o}_{o}} - \frac{[H_2PO_4^-]\bullet K_{H2PO4}\bullet[H_2O]}{[H_3O^+]}$;

$\mathbf{[H_2PO_4^-]}=\frac{\frac{[ATP^{4-}]}{[ADP^{3-}]\bullet R^{o}_{o}}}{\left(1+\frac{K_{H2PO4}\bullet[H_2O]}{[H_3O^+]}\right)}$ **;ΔG=ΔG°+R•T•ln(R°$_o$•{$\mathbf{H_2O}$]**$\left(1+\frac{K_{H2PO4}\bullet[H_2O]}{[H_3O^+]}\right)$**)=36.99 kJ/mol**

$ATP^{4-} + H_2O \Leftrightarrow ADP^{3-} + H_2PO_4^-$; **K°= 220409** **; ΔG° = -30.500 kJ/mol**

Calculate the free energy Δ**G** required to synthesize ATP^{4-} in a rat **hepatocyte**.

14. **Daily ATP Utilization by Human Adults**.

(**a**) A total of **30.5 kJ/mol** of free energy Δ**G** is needed to synthesize ATP^{4-} from ADP^{3-} and $H_2PO_4^-$ when the **reactants** and **products** are at **1 M** concentration (standard state). Because the actual **physiological** concentrations of ATP^{4-}, ADP^{3-}, and $H_2PO_4^-$ are not **1 M**, the free energy Δ**G** required to synthesize ATP^{4-} under physiological conditions is different from Δ**G°**. Calculate the free energy Δ**G** required to synthesize ATP^{4-} m the human **hepatocyte** when the physiological concentrations of
ATP^{4-}, ADP^{3-}, ($H_2PO_4^-$+ HPO_4^{2-}) are **3.5,1.50**, **5.0 mM** and **pH=7.36**, respectively, at **37°C**.

$\mathbf{K^{\circ}_{H2PO4}}=\frac{[HPO_4^{2-}]\bullet[H_3O^+]}{[H_2PO_4^-]\bullet[H_2O]}$; $\mathbf{P_i = 5\ mM=[H_2PO_4^-]+[HPO_4^{2-}]}$; $\mathbf{[H_2PO_4^-]=5 -}\frac{[H_2PO_4^-]\bullet K_{H2PO4}\bullet[H_2O]}{[H_3O^+]}$

$K^{\circ}_{H2PO4}{}^{25}$= 1.1469•10^{-9}; ΔG° = -R•T•ln(K°_{H2PO4}) = 51.034 kJ/mol at **T = 298.16 K**
$K^{\circ}_{H2PO4}{}^{37}$= 2.543489•10^{-9} at **T = 310.16 K**

$[\mathbf{H_2PO_4^-}] = \dfrac{5/1000}{\left(1+\dfrac{K_{H2PO4}\bullet[H_2O]}{[H_3O^+]}\right)}$ **;** $\Delta\mathbf{G°} = \mathbf{-R\bullet T\bullet ln}\dfrac{[ATP^{4-}]\bullet[H_2O]}{[ADP^{3-}]\bullet[H_2PO_4^-]}$ at **T = 310.16 K**

$\Delta\mathbf{G}=\Delta\mathbf{G°}+\mathbf{R\bullet T\bullet ln}\left(\dfrac{[ATP^{4-}]\bullet[H_2O]\bullet\left(1+\dfrac{K_{H2PO4}\bullet[H_2O]}{[H_3O^+]}\right)}{[ADP^{3-}]\bullet 5/1000}\right)$ **=60.3976 kJ/mol** at **T=310.16 K**

$ATP^{4-} + H_2O \Leftrightarrow ADP^{3-} + H_2PO_4^-$; **K°= 220409** **;** $\Delta\mathbf{G°}$ **= -30.500 kJ/mol**

(**b**) A **68 kg** (**150 lb**) adult requires a caloric intake of **2 000 kcal** (**8 360 kJ**) of food per day (**24 h)**. The food is metabolized and the free energy $\Delta\mathbf{G}$ is used to synthesize ATP^{4-}, which then provides energy $\Delta\mathbf{G}$ for the body's daily chemical and mechanical **work W=-**$\Delta\mathbf{G}$. Assuming that the efficiency of converting food energy **E** into ATP^{4-} is **50%**, calculate the weight $\mathbf{m_{ATP}}$ of ATP^{4-} used by a human adult in **24 h**. What percentage of the body weight does this represent?

$$\mathbf{n_{ATP} = 8360/60.397598/2 = 69.208\ mol\ ;\ m_{ATP} = n_{ATP}\bullet M_{ATP} = 35082\ g}$$

(**c**) Although adults synthesize large amounts of ATP^{4-} daily, their body weight, structure, and composition do not change significantly during this period. Explain this apparent contradiction.

15. **Rates of Turnover of □and □ Phosphates of ATP^{4-} A-O-OPO^--O-OPO^--O-OPO^--O^-** (**A-**α**-**β**-**γ**-$\mathbf{O^-}$).

If a small amount of ATP^{4-} labeled with radioactive phosphorus in the terminal position, [γ-^{32}**P**] ATP^{4-}, is added to a yeast extract, about half $^1/_2$ of the ^{32}**P** activity is found in $H_2PO_4^-$ within a few minutes, but the concentration of $[ATP^{4-}]$= const remains unchanged. Explain. If the same experiment is carried out using ATP^{4-} labeled with ^{32}**P** in the central position, [γ-^{32}**P**] ATP^{4-}, the ^{32}**P** does not appear in $H_2PO_4^-$ within such a short time. Why?

16. **Cleavage of ATP to AMP and PP_i during Metabolism**

The synthesis of the activated form of **acetate** (**acetyl-CoA**) is carried out in an **ATP**-dependent process:

$Acetate + CoA^{4-} \Leftrightarrow$ acetyl-$CoA^{4-} + H_2O$ **;** $\Delta\mathbf{G°_1}$**= 31.4 kJ/mol**

$ATP^{4-} + H_2O \Leftrightarrow AMP^{2-} + PP_i^{2-}$ **;**$\Delta\mathbf{G°_2}$ **= -45.6 kJ/mol**

$Acetate + CoA + ATP^{4-} \Leftrightarrow$ acetyl-$CoA + AMP^{2-} + PP_i^{2-}$**;**$\Delta\mathbf{G°}$ **= -14.2 kJ/mol**

(**a**) The $\Delta\mathbf{G°}$ for the **hydrolysis** of **acetyl-CoA** to **acetate** and **CoA** is **-31.4 kJ/mol** and that for **hydrolysis** of ATP^{4-} to AMP^{2-} and PP_i^{2-} is **-45.6 kJ/mol**. Calculate $\Delta\mathbf{G°}$ for the **ATP-dependent** synthesis of **acetyl-CoA**.

(**b**) Almost all cells contain the enzyme inorganic **pyro-phosphates**, which catalyzes the **hydrolysis** of PP_i^{2-} to $H_2PO_4^-$. What effect does the presence of this enzyme have on the synthesis of **acetyl-CoA**? Explain!

$H_2PO_3\text{-}O\text{-}O_3PH_2^{2-} + H_2O \Leftrightarrow 2\ H_2PO_4^-$**;**$\Delta\mathbf{G°_{PP}}$**=-19kJ/mol**;$\Delta\mathbf{G°_{total}}=\Delta\mathbf{G°}+\Delta\mathbf{G°_{PP}}$**=-33.2kJ/mol**

$H_2PO_3\text{-}O\text{-}O_3PH_2^{2-} + 3\ H_2O \Leftrightarrow 2\ HPO_4^{2-} + 2\ H_3O^+$**;**$\Delta\mathbf{G°_{PPH}}$ **= 107.21 kJ/mol**

17. **Energy for H_3O^+ Pumping** The parietal cells of the stomach lining contain membrane "pumps" that transport hydrogen ions $\mathbf{H_3O^+}$ from the **cytosol** of these cells (**pH_{plasma} 7.36**) into the **stomach**, contributing to the acidity of **gastric juice** (**$pH_{stomach}$ 1.2**). Calculate the free energy required to transport **1 mol** of hydrogen $\mathbf{H_3O^+}$ ions through these pumps. (Hint: See Oxidative Phosphorylation.)

Assume a temperature of **37 °C** or **T = 310.16 K**.

$H_3O^+_{plasma} \Leftrightarrow H_3O^+_{stomach}$**;** $\mathbf{K_{eq}} = [H_3O^+_{stomach}]/[H_3O^+_{plasma}] = 10^{-pHplasma}/10^{-pHstomach}$ **= $1.445\bullet10^6$**

$\Delta\mathbf{G°} = \mathbf{-R\bullet T\bullet ln(K_{eq}) = -36.577\ kJ/mol}$

18. **Standard Reduction Potentials** The **standard reduction potential**, **E°**, of any **RedOx** pair is defined for the half-cell reaction in equilibrium of each **RedOx system**:

Oxidizing agent^{n+} + $n_{electrons}^-$ ⇔reducing agent

The **E°** values for the **NAD^+/NADH** and **pyruvate/lactate** conjugate **RedOx**pairs are **-0.113** and **0.2291 V**, respectively but $\mathbf{E°_{37}}$**: -0.059** and **0.3193 V**.

(**a**) Which conjugate pair has the greater tendency to lose electrons? Explain.

(**b**) Which is the stronger oxidizing agent? Explain.

(**c**) Beginning with **1 M** concentrations of each **reactant** and **product** at **pH 7.36**, in which direction will the following reaction proceed?

Pyruvate$^-$ + H_3O^+ + NADH => lactate$^-$ + NAD^+ + H_2O ;($E°_{NADH}$ - $E°_{pyruvate}$)•F•2 = $\Delta G°$ = -73.000 kJ/mol

$$\Delta G = \Delta G° + R•T•\ln\left(\frac{[\text{lactate}^-]•[NAD^+]•[H_2O]}{[\text{pyruvate}^-]•[NADH]•[H_3O^+]}\right)$$ = **-18.957 kJ/mol** favorable direction of reaction

(**d**) What is the standard free-energy change ($\Delta G°$) at **37 °C** for the conversion of **pyruvate** to **lactate**

(**e**) What is the equilibrium constant (K_{eq}) for this reaction?

$$K_{eq} = \frac{[\text{lactate}^-]•[NAD^+]•[H_2O]}{[\text{pyruvate}^-]•[NADH]•[H_3O^+]}$$ =**6.149•10^{12}; [H_3O^+] •K_{eq}**= $\frac{[\text{lactate}^-]•[NAD^+]•[H_2O]}{[\text{pyruvate}^-]•[NADH]}$ =**268417**

19. **Energy Span of the Respiratory Chain** Electron **e$^-$** transfer in the mitochondrial **respiratory chain** may be represented by the net reaction equation

2 NADH + 2 H_3O^+ + O_2=> 2 NAD^+ + 4 H_2O ($E°_{NADH}$ - $E°_{O2}$)•F•4 = $\Delta G°$ = -552.74 kJ/mol

(**a**) Calculate the value of $\Delta E°$ for the net reaction of mitochondrial electron **e$^-$** transfer at **37°C**.

(**b**) Calculate $\Delta G°$ for this reaction. **$\Delta E° = E°_{NADH} - E°_{O2}$ = -0.059-1.3732 = -1.4322 V**

(**c**) How many **nATP** molecules can theoretically be generated by this reaction if the free energy of **ATP** synthesis under cellular conditions is **52 kJ/mol**? **n = 10.63**

20. **Dependence of Electromotive Force on Concentrations**

Calculate the electromotive force **EMF** (in volts **V**) registered by an electrode immersed in a solution containing the following mixtures of **NAD^+** and **NADH** at **pH 7.36** and **37 °C**, with reference to a half-cell of **E° 0.00 V**.

NADH + H_2O ⇔ NAD^+ + H_3O^+ + 2e$^-$ E_{37} = -0.0590 V (David Harris)

$$\text{EMF} = E = E_{37} + R•T/F/2•\ln\left(\frac{[NAD^+]•[H_2O]}{[NADH]•[H_3O^+]}\right)$$ = **-0.36983 V**

(**a**) **1.00 mM NAD^+** and **10.0 mM NADH; E = -0.36983 V**

(**b**) **1.00 mM NAD^+** and **1.00 mM NADH; E = -0.33906 V**

(**c**) **10.0 mM NAD^+** and **1.00 mM NADH ; E = -0.30829 V**

21. **Electron Affinity of Compounds** List the following substances in order of increasing □ tendency to accept electrons **e$^-$** at **pH = 7.36** by **RedOx potential E_o** values:

O_{2g}+4 H_3O^++ 4 e$^-$= 6 H_2O Suchotina**;** **E_o = 0.8130 V ; E_{37} = 1.3732 V**

O_{2aq}+2H_3O^++2e$^-$=H_2O_{2aqua}+2H_2O University Alberta**; E_o = 0.2336 V ; E_{37} = 0.7937 V**

oxalo-acetate^{2-}+2H_3O^+ +2e$^-$= Malate^{2-}+2H_2O CRC **;** **E_o = -0.2225 V ; E_{37} = 0.3376 V**

O_{2g}+ e$^-$ = O^-_{2aq}Suchotina**;** **E_o = -0.2355 V ; E_{37} = -0.2355 V**

$NADP^+$+H_3O^+ +2e$^-$= NADPH + H_2O CRC **;** **E_o = -0.3429 V ; E_{37} = -0.0629 V**

α-Ketoglutarate+CO_2+2H_3O^++2e$^-$= isocitrate+2H_2O ; E_o = -0.4283 V ; E_{37} = 0.13185 V

(**a**) α-keto-glutarate + CO_2 (yielding iso-citrate);

(**b**) oxalo-acetate;

(**c**) **O_2** ;

(**d**) **$NADP^+$**.

22. **Direction of Oxidation-Reduction Reactions**

Which of the following reactions would you expect to proceed in the direction shown under **standard conditions pH = 7.36** and **37°C**, assuming that the appropriate **enzymes** are present to catalyze them?

($E°_{Red}$-$E°_{Ox}$)•F•n = $\Delta G°$ kJ/mol ;

$\Delta G = \Delta G^\circ + R \cdot T \cdot \ln\left(\frac{[\text{oxaloacetate}^{2-}] \bullet [NADH] \bullet [H_3O^+]}{[\text{malate}^{2-}] \bullet [NAD^+] \bullet [H_2O]}\right)$ = **10.053 kJ/mol** unfavorable direction => for (**a**)

(**a**) $Malate^{2-} + NAD^+ + H_2O \Rightarrow$ oxalo-$acetate^{2-} + NADH + H_3O^+$; $(E^\circ_{malate} - E^\circ_{NAD+}) \cdot F \cdot 2 = \Delta G^\circ = 53.756$ **kJ/mol**

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$\Delta G = \Delta G^\circ - R \cdot T \cdot \ln(10^{-7.36}) = 0.568$ **kJ/mol** <=direction favorable to left for (**b**)

(**b**) aceto-$acetate^-$ + NADH + H_3O^+ => β-hydroxy-$butyrate^-$ + NAD^+ + H_2O; $\Delta G^\circ = -43.135$ **kJ/mol**

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$\Delta G = \Delta G^\circ - R \cdot T \cdot \ln(10^{-7.36}) =$ **-29.298 kJ/mol** => direction favorable to right

(**c**) $Pyruvate^-$ + NADH + H_3O^+ => $lactate^-$ + NAD^+ + H_2O ; $\Delta G^\circ = -73.001$ **kJ/mol** =>direction

(**d**) $Pyruvate^-$ + β-hydroxy-$butyrate^-$ => $lactate^-$ + aceto-$acetate^-$; $\Delta G^\circ = -29.866$ **kJ/mol** => direction

(**e**) $Malate^-$ + $pyruvate^-$ => oxalo-$acetate^-$ + $lactate^-$; $\Delta G^\circ = 3.526$ **kJ/mol** <= direction

(**f**) Acetaldehyde + $succinate^{2-}$ => ethanol + $fumarate^{2-}$; $\Delta G^\circ = 46.447$ **kJ/mol** <= direction