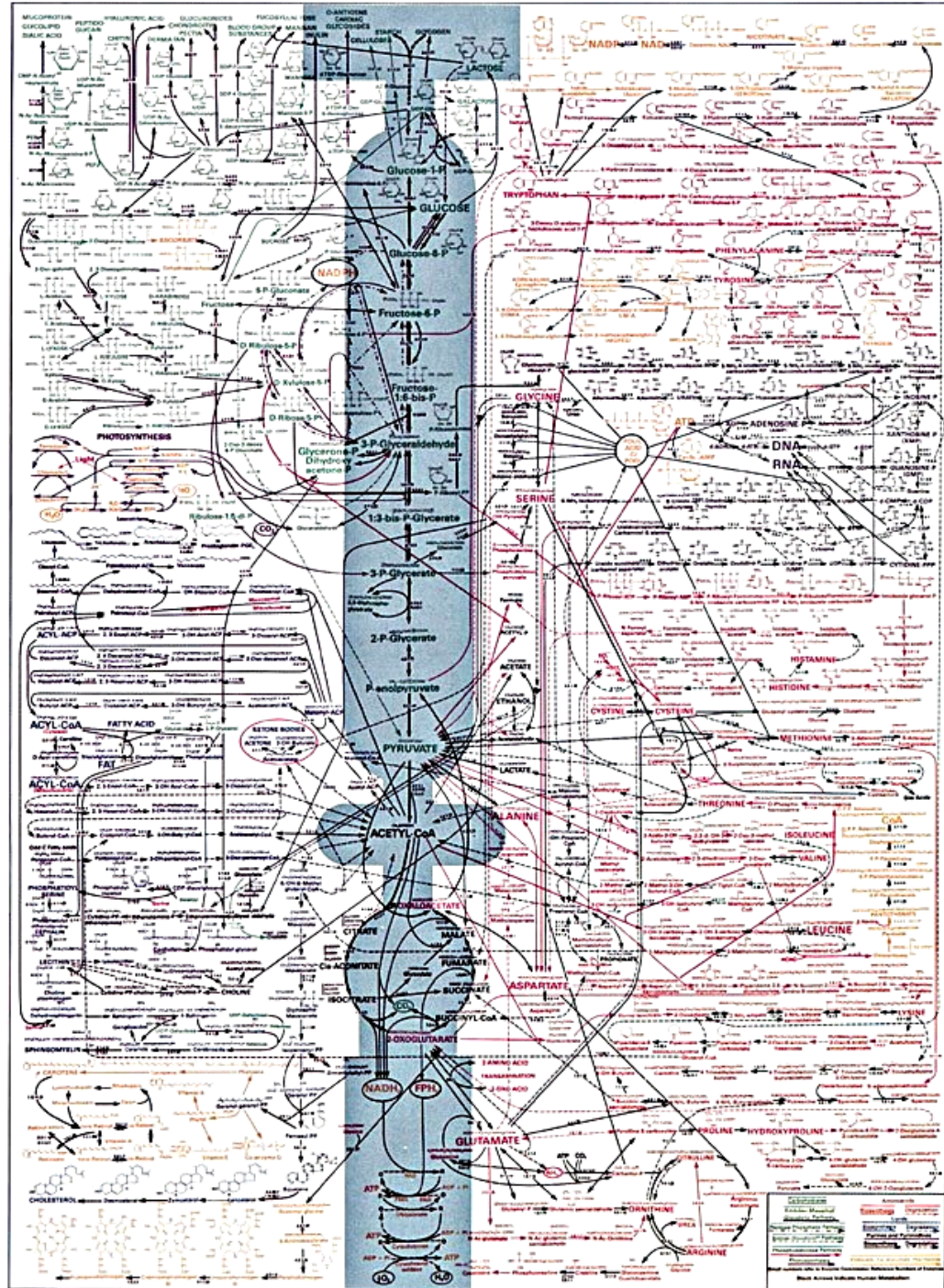


Introduction to Bioenergetics

- Thermodynamics
- Free energy
- Equilibrium
- Coupled reactions
- Steady state
- High energy bonds
- Redox potential



Thermodynamics Review

First Law: *For any physical or chemical change, the total amount of energy in the universe remains constant. Energy may change form or be transported, but it cannot be created or destroyed.*

Second Law: *In all natural processes, the entropy of the universe (total S) increases.*

- **Key terms we will deal with in this chapter:**

- **System / Surroundings / Universe**

- **Energy, Heat, Work** - influence on system

$$1 \text{ J} = (\text{Kg-m} / \text{sec}^2) ; 1 \text{ cal} = 4.184 \text{ J}$$

- **Enthalpy (H) and Entropy (S)**

$$H = E + PV \text{ or } \Delta H = \Delta E + P\Delta V$$

$$S = k \ln W \text{ or } \Delta S = k \ln(W_f / W_i)$$

Joule (J)

$$1 \text{ J} = 1 \text{ kg} \cdot \text{m}^2 \cdot \text{s}^{-2} \quad 1 \text{ J} = 1 \text{ C} \cdot \text{V}(\text{coulomb volt})$$

$$1 \text{ J} = 1 \text{ N} \cdot \text{m} \text{ (newton meter)}$$

Calorie (cal)

1 cal heats 1 g of H₂O from 14.5 to 15.5°C

$$1 \text{ cal} = 4.184 \text{ J}$$

Large calorie (Cal)

$$1 \text{ Cal} = 1 \text{ kcal} \quad 1 \text{ Cal} = 4184 \text{ J}$$

Avogadro's number (N)

$$N = 6.0221 \times 10^{23} \text{ molecules} \cdot \text{mol}^{-1}$$

Coulomb (C)

$$1 \text{ C} = 6.241 \times 10^{18} \text{ electron charges}$$

Faraday (F)

$$1 \text{ F} = N \text{ electron charges}$$

$$1 \text{ F} = 96,485 \text{ C} \cdot \text{mol}^{-1} = 96,485 \text{ J} \cdot \text{V}^{-1} \cdot \text{mol}^{-1}$$

Kelvin temperature scale (K)

$$0 \text{ K} = \text{absolute zero} \quad 273.15 \text{ K} = 0^\circ\text{C}$$

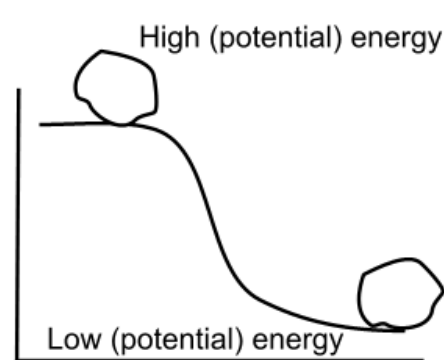
Boltzmann constant (k_B)

$$k_B = 1.3807 \times 10^{-23} \text{ J} \cdot \text{K}^{-1}$$

Gas constant (R)

$$R = Nk_B \quad R = 1.9872 \text{ cal} \cdot \text{K}^{-1} \cdot \text{mol}^{-1}$$

$$R = 8.3145 \text{ J} \cdot \text{K}^{-1} \cdot \text{mol}^{-1} \quad R = 0.08206 \text{ L} \cdot \text{atm} \cdot \text{K}^{-1} \cdot \text{mol}^{-1}$$



For $R \rightarrow P$

$$\Delta E = E_p - E_r$$

ΔE will be negative
for "spontaneous event"

First law of thermodynamics:
total energy is conserved in ordinary reactions

Energy is exchanged, in chemistry, in 2 main ways,
heat and work (electrical work will be dealt with later).

In a chemical reaction

$$\Delta E = q - w$$

(ie heat added TO the system - work done BY the system)

heat change is basically a measure of difference in bond
energies between reactants and products.

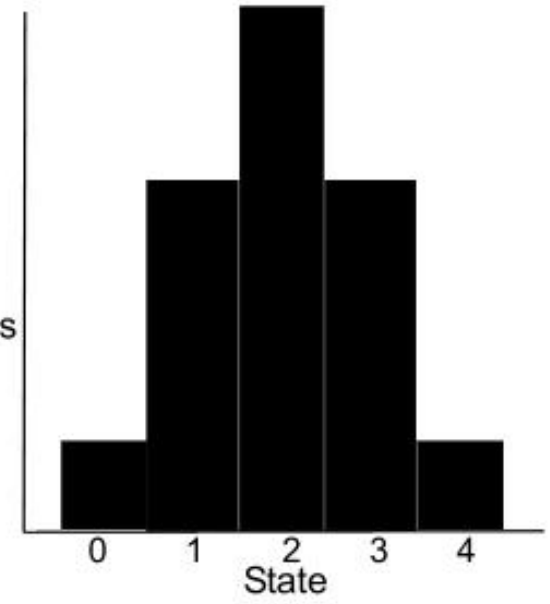
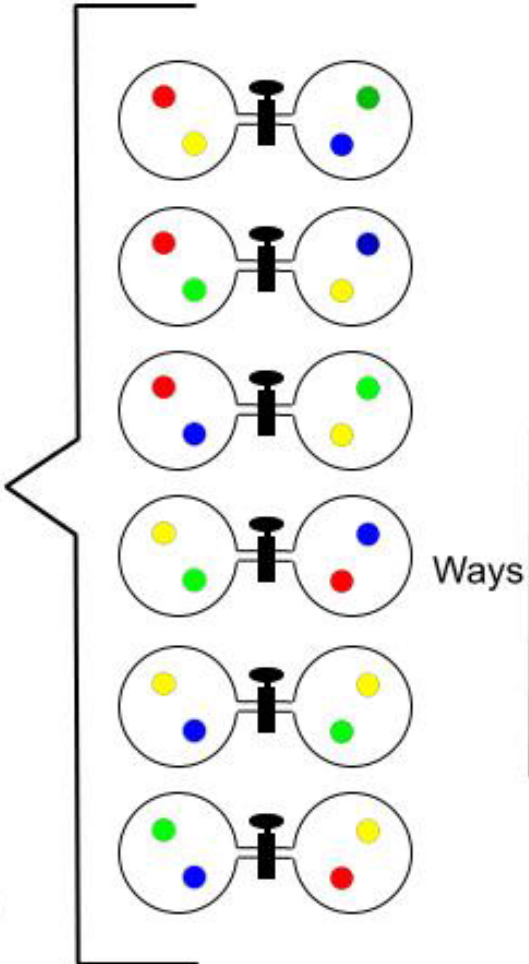
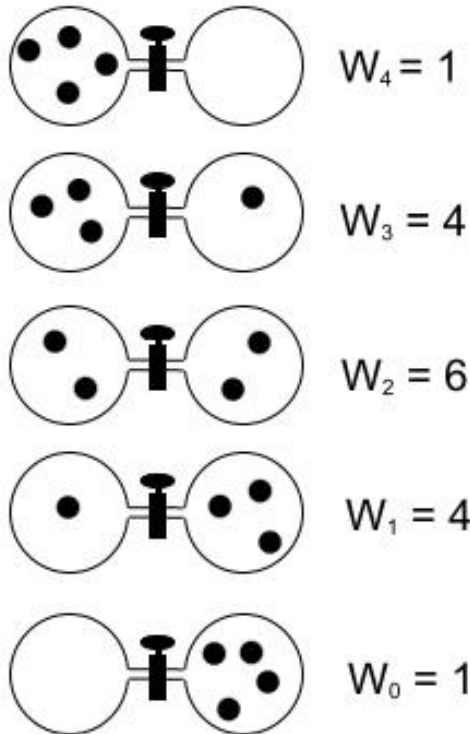
If only $P\Delta V$ work is possible, then $\Delta E = q - w = q - P\Delta V$

Define enthalpy as $\Delta H = \Delta E + P\Delta V$ that is the heat release
observed due to changes in internal energy and PV work .

In biological reactions, $P\Delta V$ work is so small
we can think of ΔH as a change in internal energy,
that is bond strengths.

Entropy and States

$$W_L = \frac{N!}{L!(N-L)!}$$



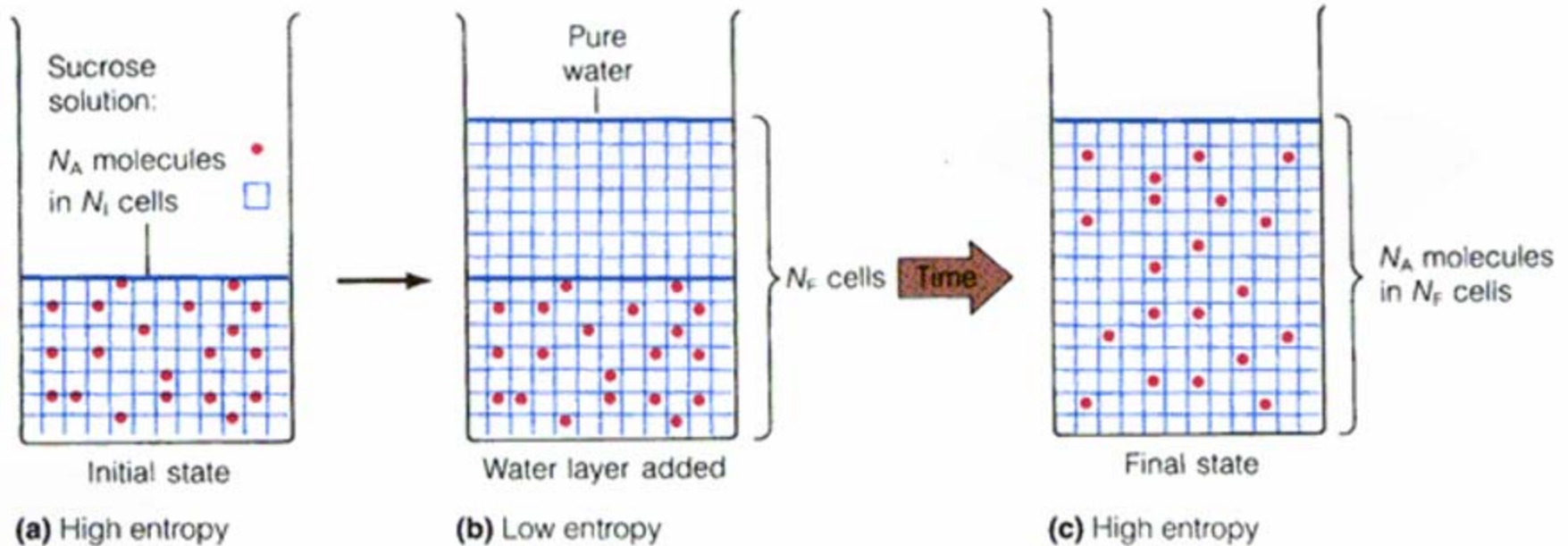
Total Ways = 1 + 4 + 6 + 4 + 1 = 16

Most common state = 6/16 of total = 37.5%. If we used 100 particles,

the most common state (50:50) would

account for 99.5% of the total. For molar numbers, the most common state is effectively the ONLY state. The magnitude of these numbers make a logarithmic definition better: $S = k_B \ln W$

Entropy of dilution



$S = k \ln W$ where $W =$ "ways" of arranging solute.
 There are N_1 ways to place first solute, $N_1 - 1$ for second etc. BUT since $N_1 \gg N_A$, these are all \approx same, $\therefore W_1 \approx N_1 \times N_1 \times N_1 \dots = N_1^{N_A}$ and $W_F = N_F^{N_A}$.
 The Δ entropy upon dilution is $S_F - S_i$

$$\Delta S = k \ln N_F^{N_A} - k \ln N_i^{N_A} = k \ln [N_F^{N_A} / N_i^{N_A}] = N_A k \ln N_F / N_i$$

For $N_A = 1$ mole, $\Delta S = R \ln N_F / N_i$ and given that $V_F \sim N_F$ etc.

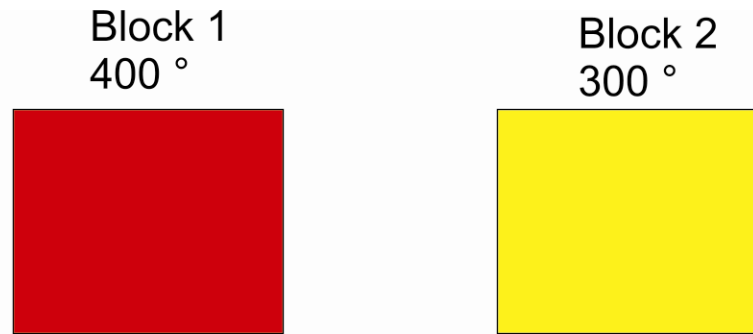
$$\Delta S = R \ln V_F / V_i$$

A similar, but more complicated derivation shows for a given solute: $\Delta S = R \ln C_F / C_i$

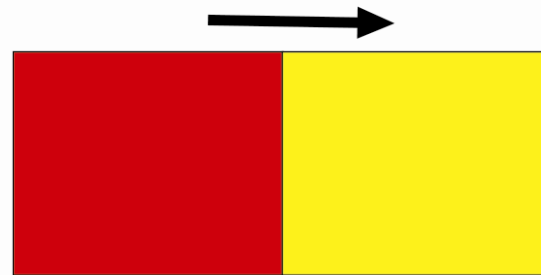
Heat and entropy

- The statistical models are almost useless in Biochem. It can be shown, though, that entropy can be measured via heat change.
- A small addition of heat to a hot system adds little entropy (already stirred up), but adding the same heat to a cold system makes a larger entropic change.
- $\Delta S \sim q/T$ the lower the absolute T, the greater entropy change
- The system we are focused on may increase or decrease entropy during a living reaction, but the entropy of the universe always increases.
- If the temperature is NOT constant, we must sum heat change over each T as: $\Delta S > \int dq/T$

An example of spontaneous reaction



800 cal flows from hot to cold



$$\Delta S_1 = -800/400$$

$$= -2 \text{ cal/deg}$$

$$\Delta S_2 = +800/300$$

$$= +2.67 \text{ cal/deg}$$

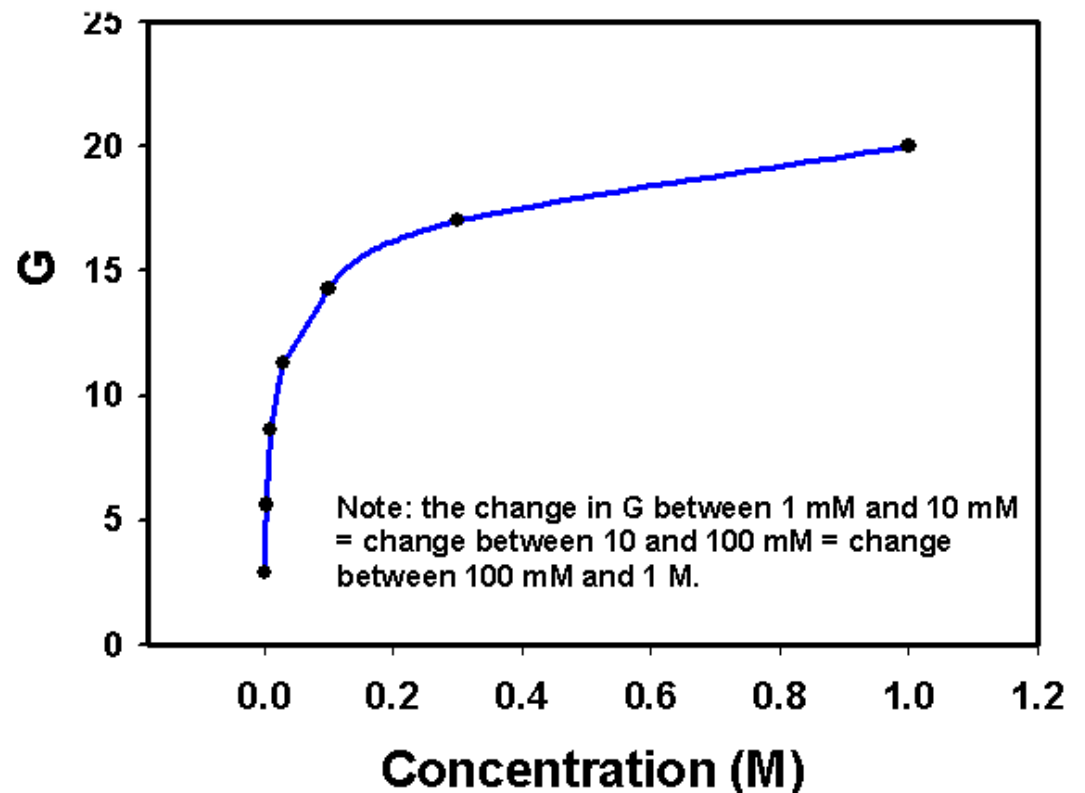
Block 1 got more ordered as it lost heat, and its entropy decreased, but Block 2 increased even more.

$$\Delta S_u = \Delta S_1 + \Delta S_2 = 0.67 \text{ cal/deg}$$

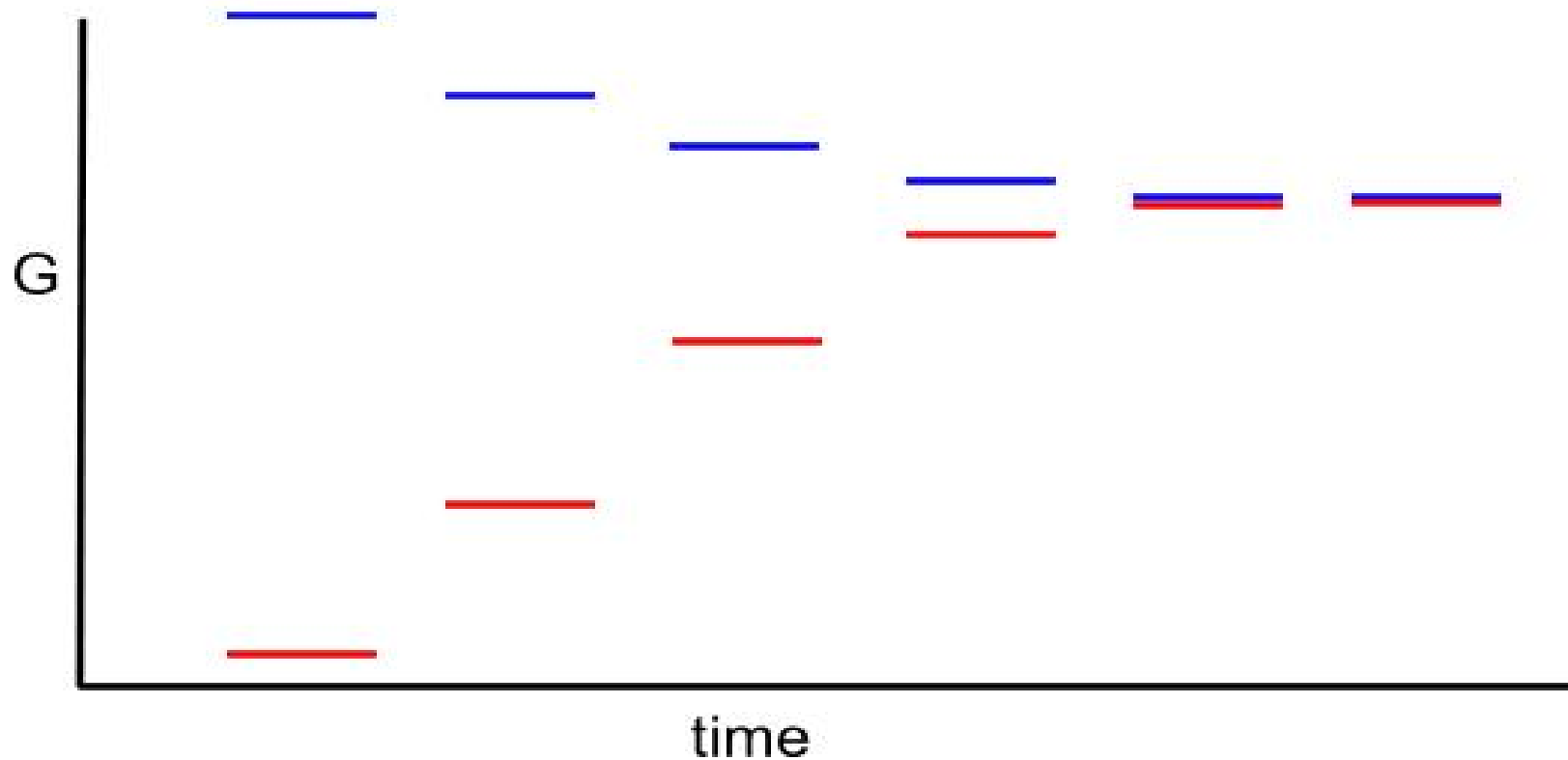
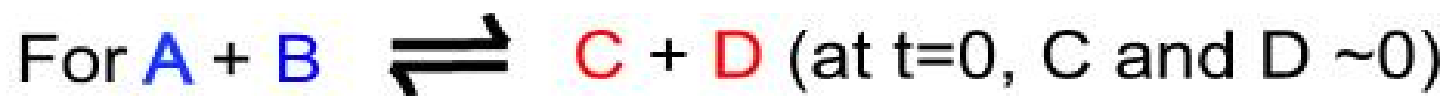
The entropy change of the universe always increases.

Free energy combines enthalpy and entropy

- $G = H - TS$ this property is non-linear with concentration (recall the logarithmic nature of the entropy)
- For any molecule, G varies with concentration.
- $G = G^0 + RT \ln C$
- $= G^0 + RT \cdot 2.303 \log C$
- $= G^0 + 5.7 \text{ KJ/mol} \log C$



As systems go to equilibrium, ΔG goes to zero



ΔG controls Equilibrium

In the system $A + B \rightleftharpoons C + D$

$$\Delta G = G_C + G_D - (G_A + G_B) = G_P - G_R$$

However, for each species $G_i = G_i^0 + RT \ln C_i$ therefore,

$$\begin{aligned} \Delta G &= G_C^0 + RT \ln C_C + G_D^0 + RT \ln C_D - (G_A^0 + RT \ln C_A + G_B^0 + RT \ln C_B) \\ &= \Delta G^0 + RT \ln [(C)(D)/(A)(B)] = \Delta G^0 + RT \ln [(C)(D)/(A)(B)] \end{aligned}$$

At $T=298$ K, and converting natural logs to base 10 we get:

$$\begin{aligned} \Delta G &= \Delta G^0 + 1.36 \text{ Kcal/mol} \log [(C)(D)/(A)(B)] \\ &= \Delta G^0 + 5.7 \text{ KJ/mol} \log [(C)(D)/(A)(B)] \end{aligned}$$

Note: we will use this expression for steady state problems.

For a system at equilibrium, $\Delta G = 0$. This connects ΔG to equilibrium distribution since the transport between reactants and products has now “settled down” and the ratio reflects K_{eq} .

$$\begin{aligned} 0 &= \Delta G^0 + 1.36 \text{ Kcal/mol} \log K_{eq} = \Delta G^0 + 5.7 \text{ KJ/mol} \log K_{eq} \text{ or} \\ \Delta G^0 &= -1.36 \text{ Kcal/mol} \log K_{eq} = -5.7 \text{ KJ/mol} \log K_{eq} \end{aligned}$$

so $\Delta G^\circ = -RT \ln K_{eq}$; $\Delta G^\circ = -2.3RT \log K_{eq}$

Note: $RT = (8.314\text{J/m-K})(298\text{K}) = 2.48 \text{ kJ/m}$

$\Delta G^\circ = -5.7 \text{ kJ/m}(\log K_{eq})$ at 25C

K_{eq}	$\Delta G^\circ(\text{kJ} \cdot \text{mol}^{-1})$ 25°C
10^6	- 34.3
10^4	- 22.8
10^2	- 11.4
10^1	- 5.7
10^0	0.0
10^{-1}	5.7
10^{-2}	11.4
10^{-4}	22.8
10^{-6}	34.3

Gibbs Free Energy and Spontaneity

Note: the standard free-energy tells us the direction a reaction will go when the initial concentration of each component is 1M, pH = 7, T = 298K, and P = 1 atm.

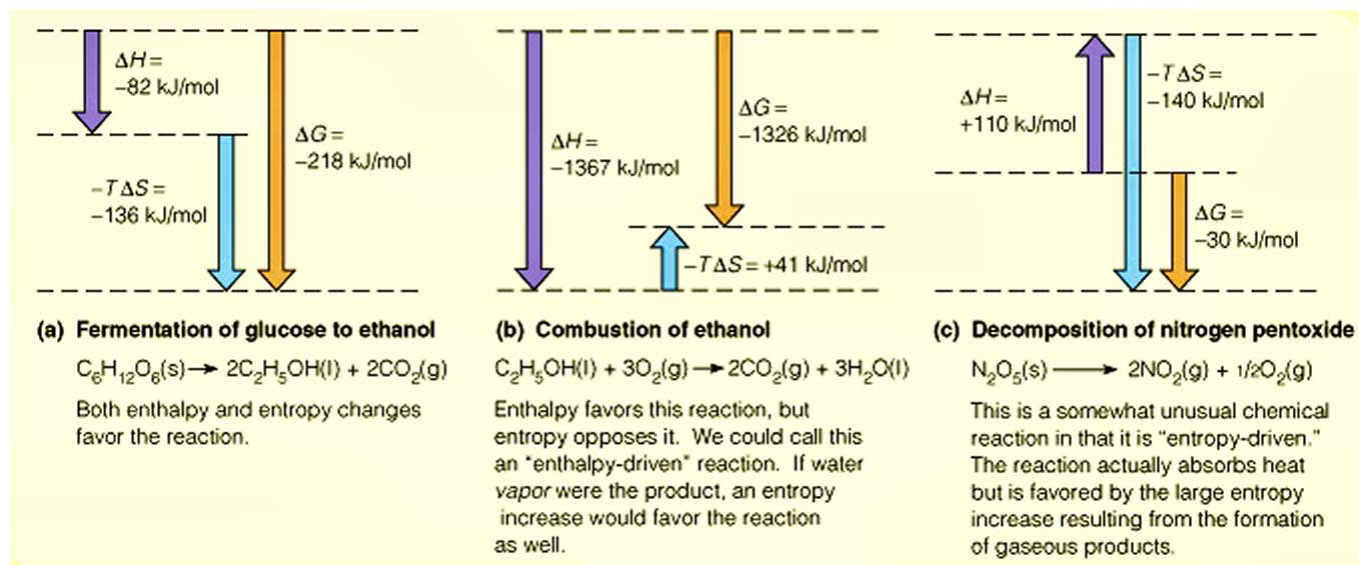
Spontaneous processes are favored if they are energetically "down hill"

(i.e. exothermic, **neg. ΔH**) and have an increase in entropy (**pos. ΔS**)

($\Delta G < 0$ - exergonic / "spontaneous")

($\Delta G = 0$ - at equilibrium)

($\Delta G > 0$ - endergonic / "non-spontaneous")



Free energies of formation have been worked out for many biologically important molecules

Compound	$-\Delta G_f^\circ$ (kJ \cdot mol $^{-1}$)		
Acetaldehyde	139.7	Glucose-6-phosphate $^{2-}$	1760.2
Acetate	369.2	Glyceraldehyde-3-phosphate $^{2-}$	1285.6
Acetyl-CoA	374.1 ^a	H $^+$	0.0
<i>cis</i> -Aconitate $^{3-}$	920.9	H $_2$ (g)	0.0
CO $_2$ (g)	394.4	H $_2$ O(ℓ)	237.2
CO $_2$ (aq)	386.2	Isocitrate $^{3-}$	1160.0
HCO $_3^-$	587.1	α -Ketoglutarate $^{2-}$	798.0
Citrate $^{3-}$	1166.6	Lactate $^-$	516.6
Dihydroxyacetone phosphate $^{2-}$	1293.2	L-Malate $^{2-}$	845.1
Ethanol	181.5	OH $^-$	157.3
Fructose	915.4	Oxaloacetate $^{2-}$	797.2
Fructose-6-phosphate $^{2-}$	1758.3	Phosphoenolpyruvate $^{3-}$	1269.5
Fructose-1, 6-bisphosphate $^{4-}$	2600.8	2-Phosphoglycerate $^{3-}$	1285.6
Fumarate $^{2-}$	604.2	3-Phosphoglycerate $^{3-}$	1515.7
α -D-Glucose	917.2	Pyruvate $^-$	474.5
		Succinate $^{2-}$	690.2
		Succinyl-CoA	686.7 ^a

Keq can be calculated from free energies of formation

example:

find Keq for hydrolysis of gly-ala + H₂O ⇌ gly + ala

tables show G_f⁰ as:

gly = -87.7 Kcal/mol; ala = -87.3 Kcal/mol;

gly-ala = -114.7 Kcal/mol; H₂O = -56.2 Kcal/mol

$$\Delta G^0 = G^0_P - G^0_R = (-87.7 - 87.3) - (-114.7 - 56.2) = -4.1 \text{ Kcal/mol}$$

from $\Delta G^0 = -1.36 \text{ Kcal/mol} \log \text{Keq}$

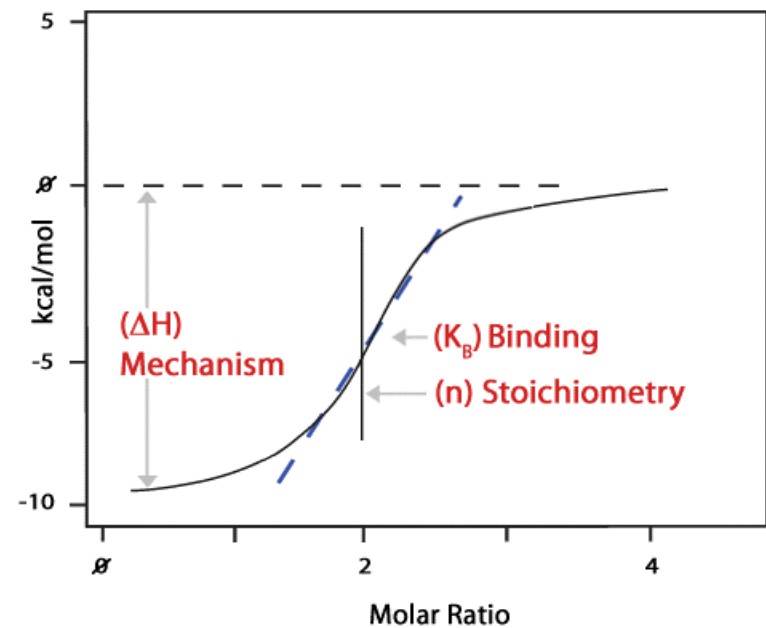
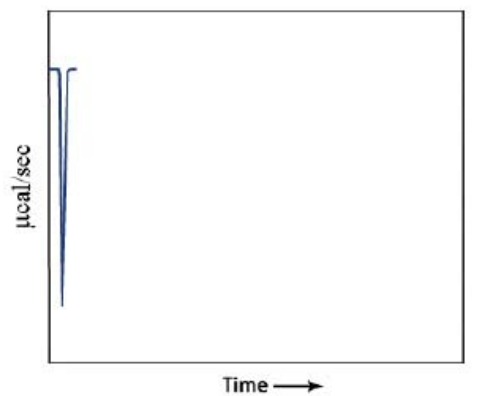
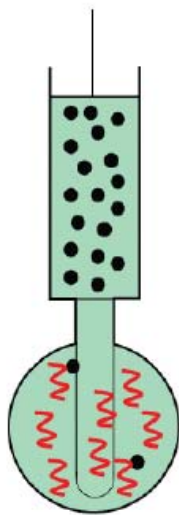
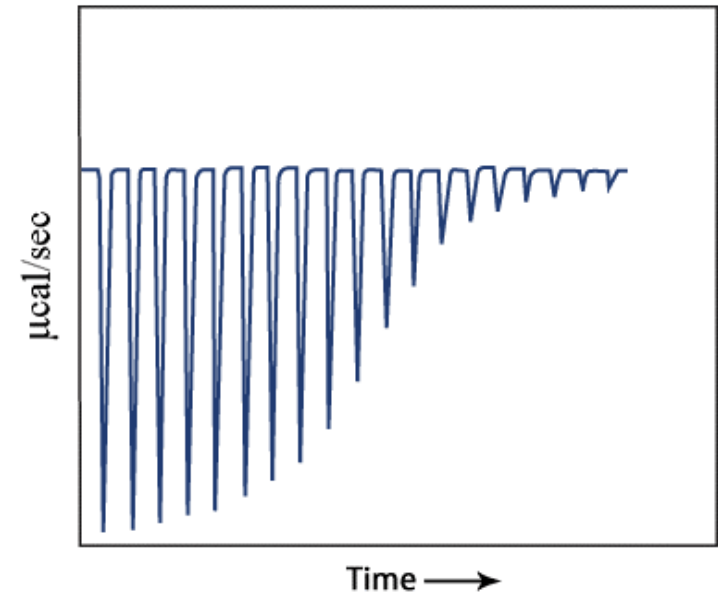
$$-4.1 \text{ Kcal/mol} = -1.36 \text{ Kcal/mol} \log \text{Keq}$$

$$3.01 = \log \text{Keq}; \quad \text{Keq} = 10^{3.01} = 1034 = 1.03 \times 10^3$$

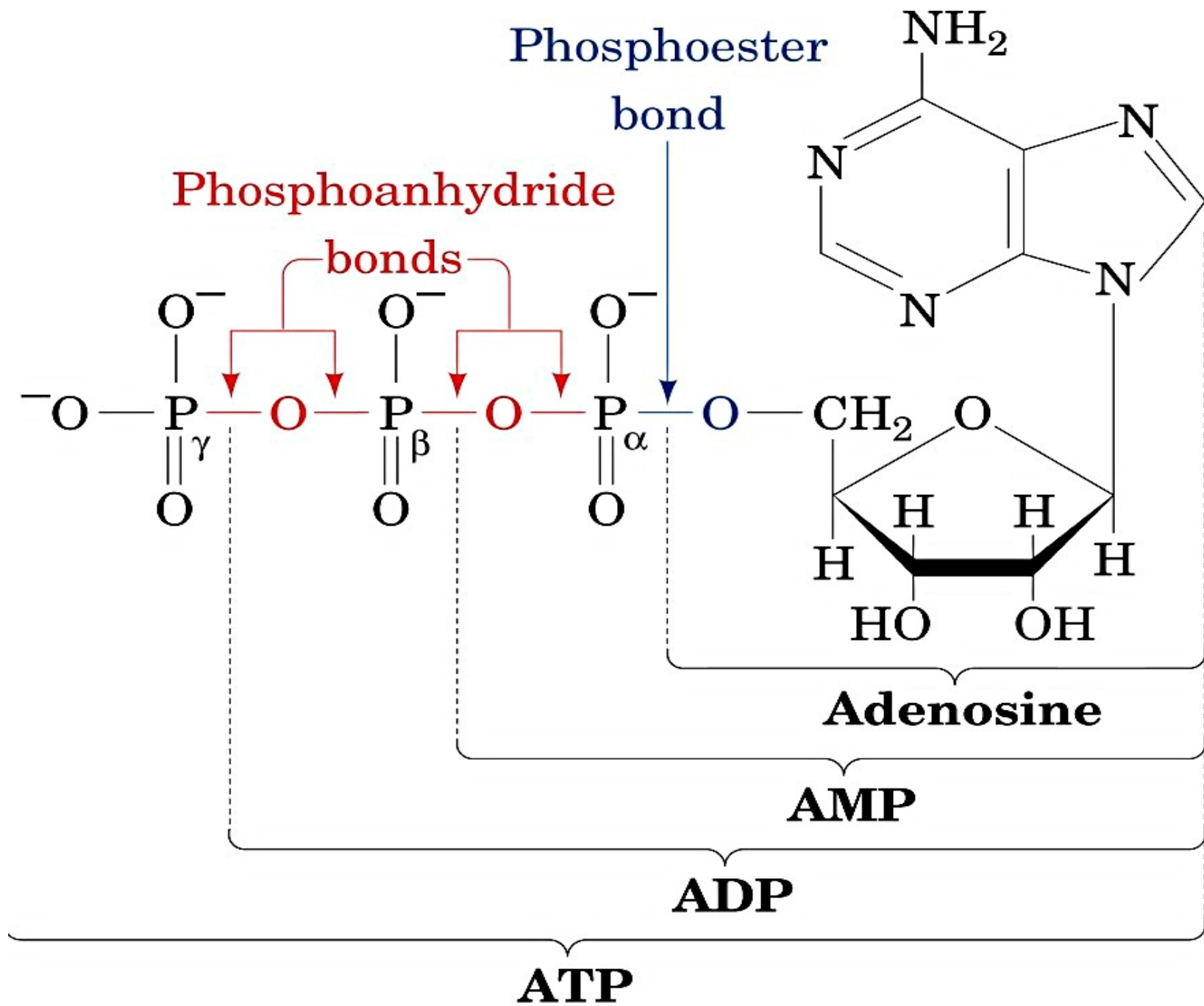
Isothermal Calorimetry



A very useful tool in drug discovery and design.

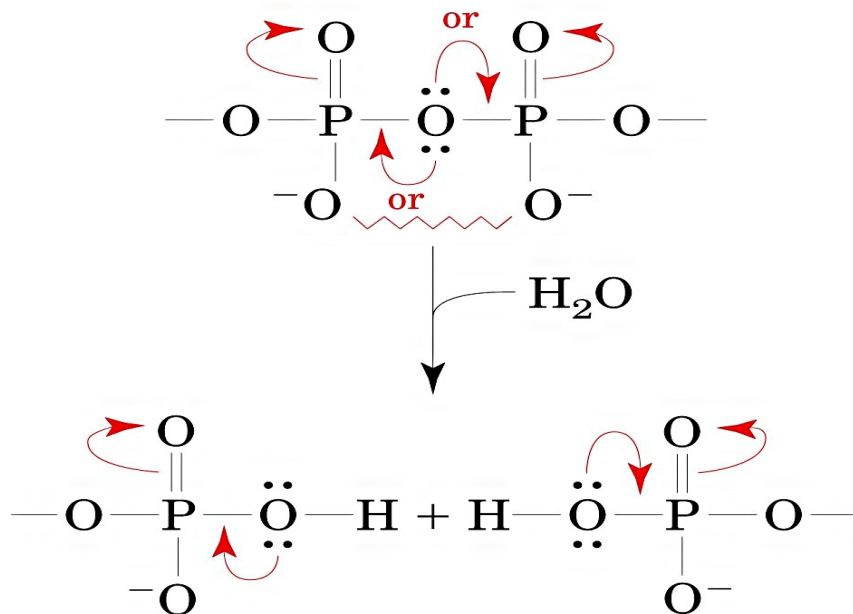
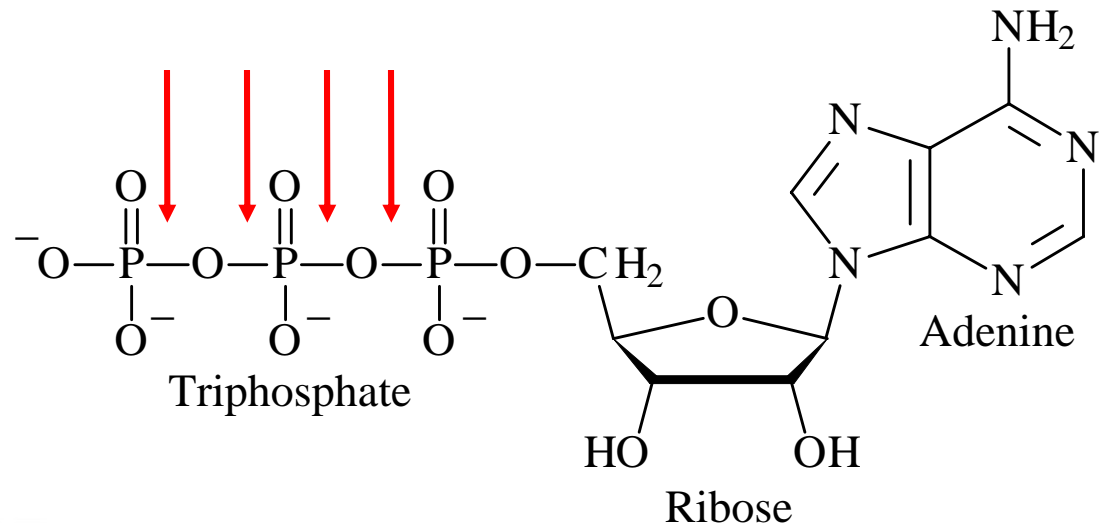


“High energy” bonds useful in
biology



-Reasons for “high-energy”:

- - charge separation
- - product stabilization
- - greater hydration



Other “High Energy” compounds

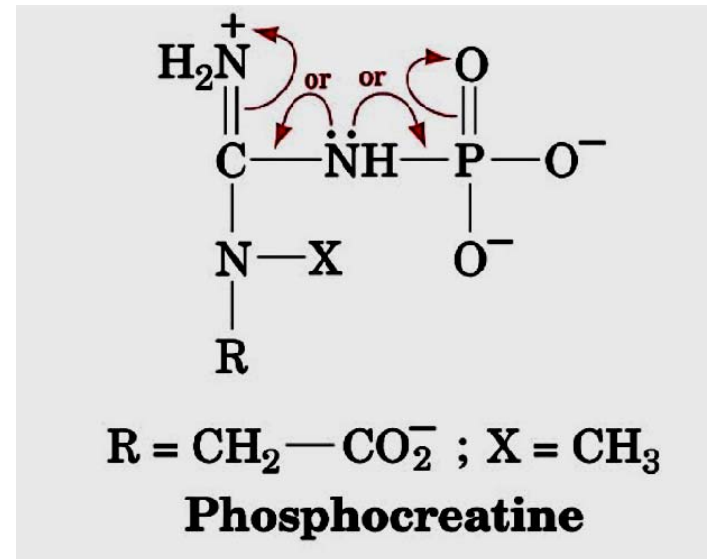
Other “High Energy” phosphorylated compounds:

PEP - enol phosphates

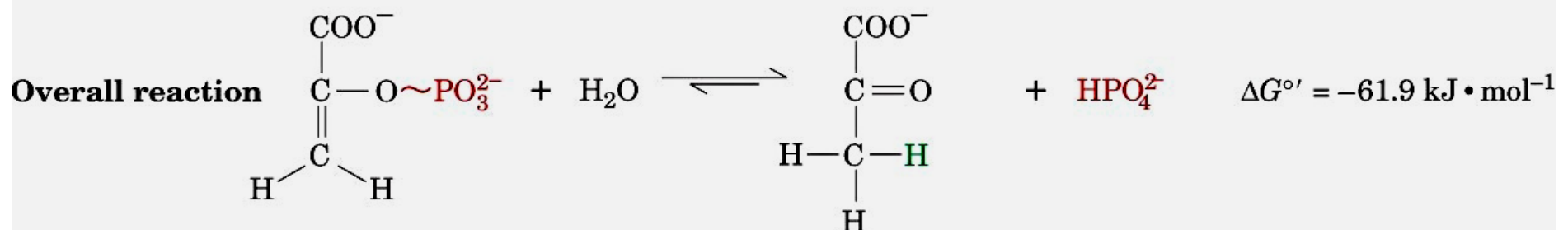
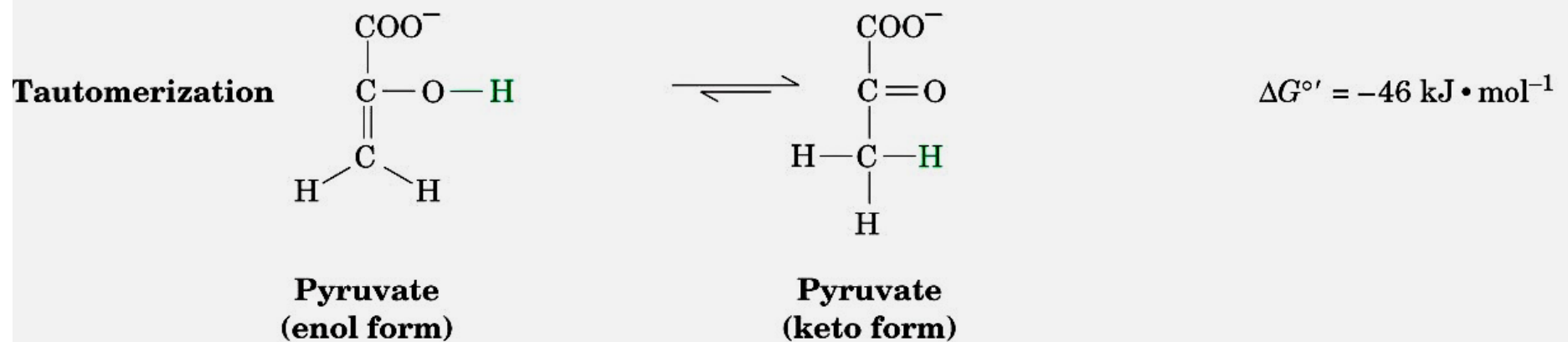
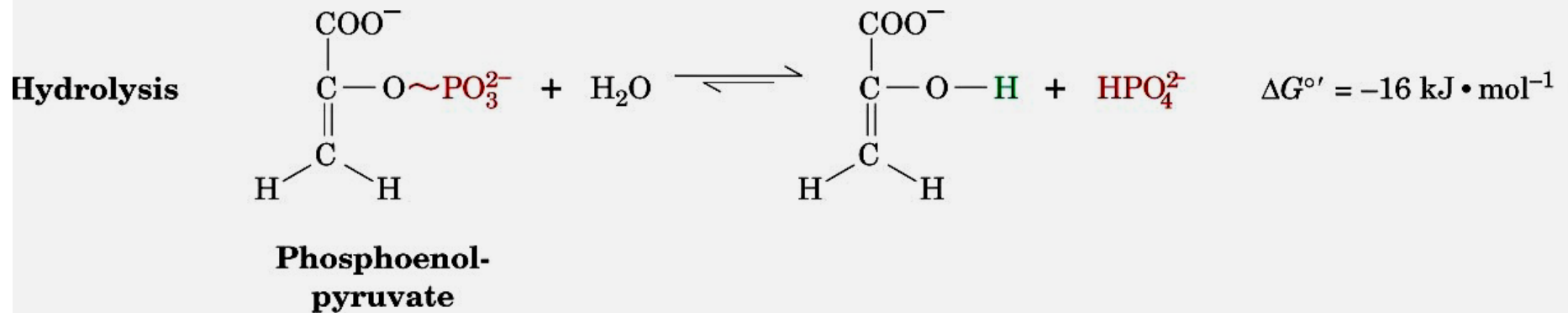
1,3-BPG - acyl phosphate (mixed anhydride)

Phosphocreatine

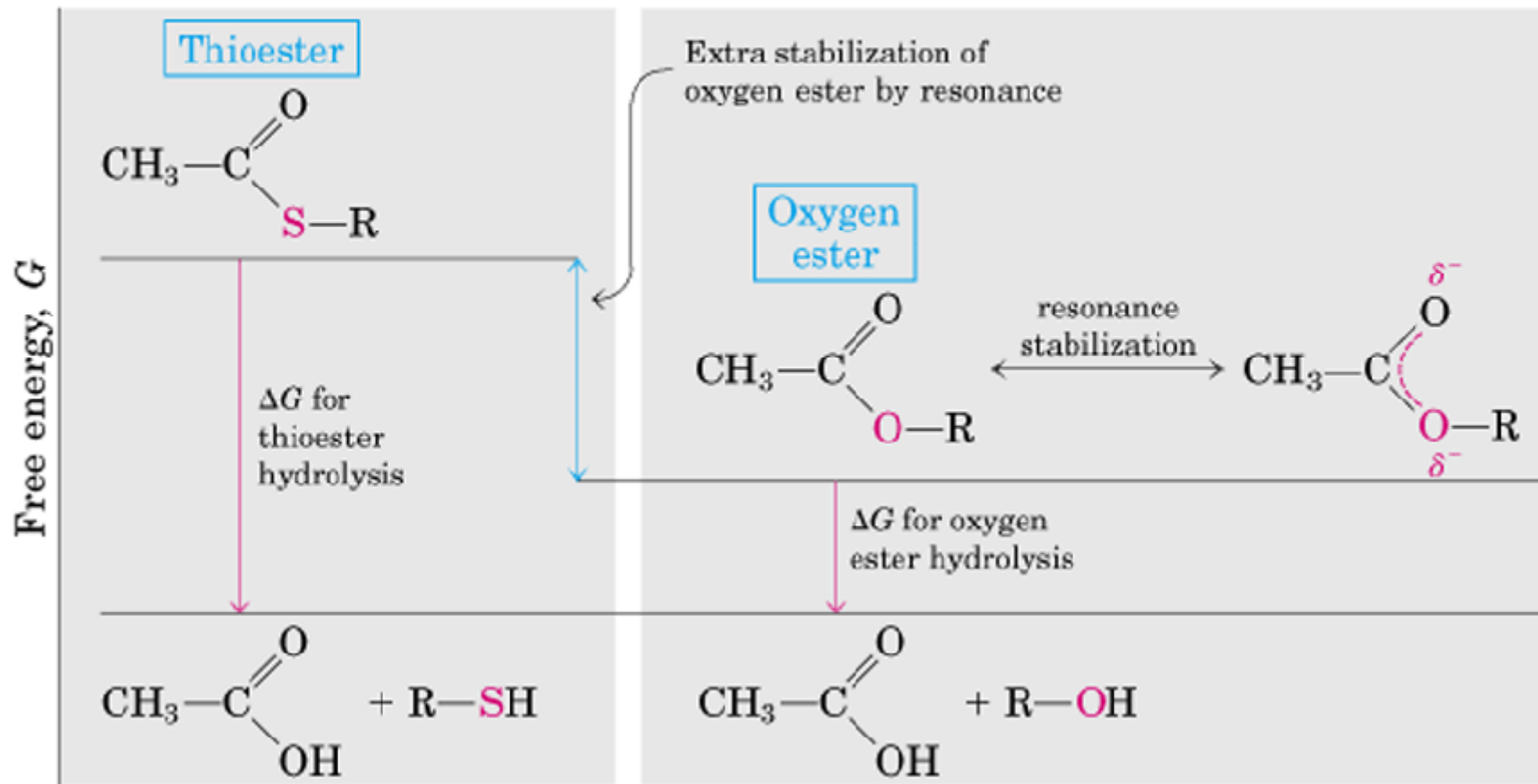
(phosphoguanidines)



Other “High Energy” compounds: Thioesters (acetyl-CoA)



Thioesters are "high energy"

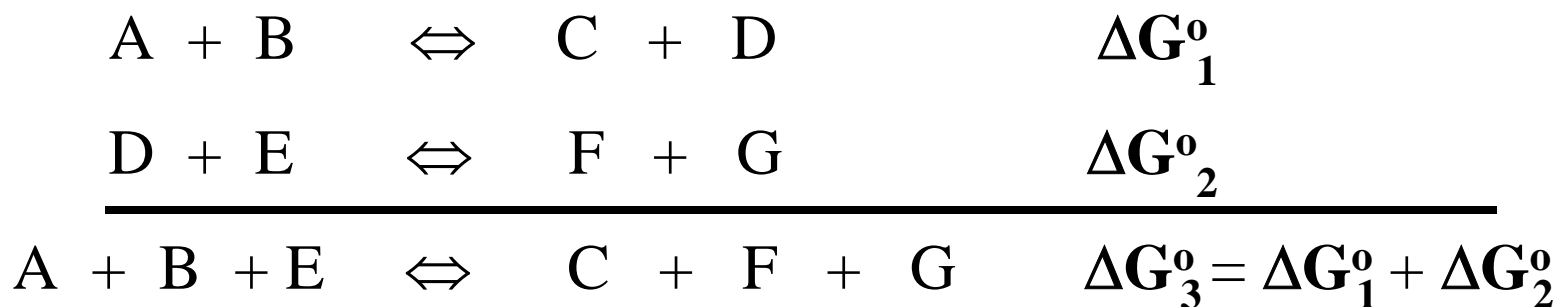


Compound	ΔG (kJ · mol ⁻¹)
Phosphoenolpyruvate	-61.9
1,3-Bisphosphoglycerate	-49.4
Acetyl phosphate	-43.1
Phosphocreatine	-43.1
PP _i	-33.5
ATP (→ AMP + PP_i)	-32.2
ATP (→ ADP + P_i)	-30.5
Glucose-1-phosphate	-20.9
Fructose-6-phosphate	-13.8
Glucose-6-phosphate	-13.8
Glycerol-3-phosphate	-9.2

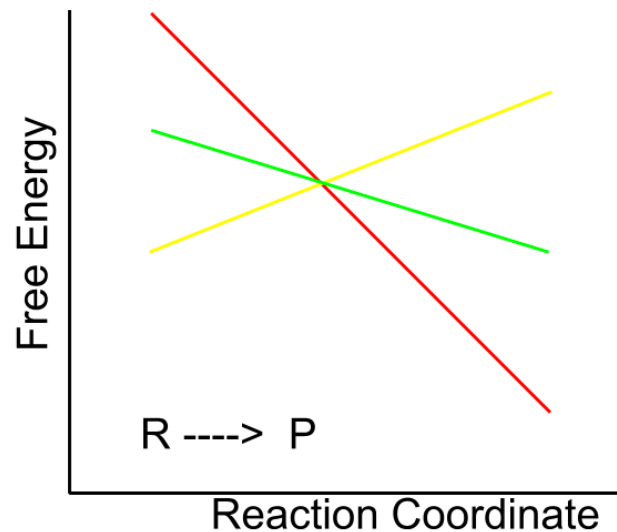
Source: Jencks, W.P., in Fasman, G.D. (Ed.), Handbook of Biochemistry and Molecular Biology (3rd ed.), Physical and Chemical Data, Vol. I, pp. 296–304, CRC Press (1976).

Coupled Reactions

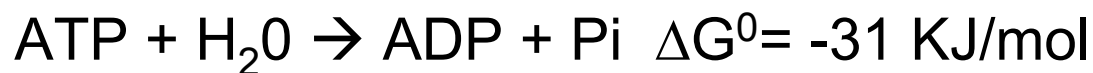
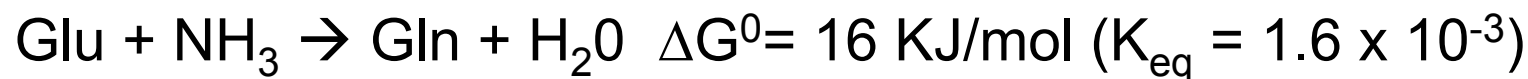
Using a favorable reaction to drive an unfavorable one.



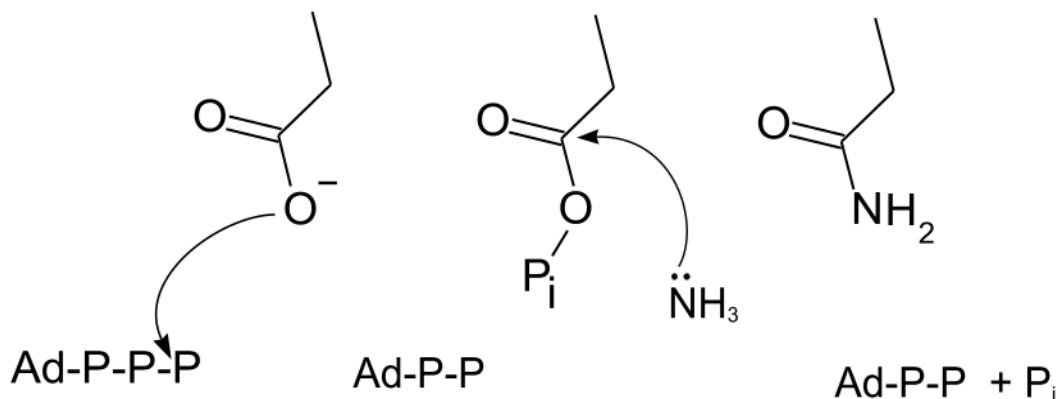
Note that the new, coupled, reaction has the desired products but that K_{eq} for reactions 1 and 2 has not changed. We write a NEW reaction which gives us what we want.



Example: Gln synthesis



This coupling involves the transfer of P_i from ATP to the Glu side chain to create a high energy mixed anhydride which is attacked by the ammonia nucleophile to release P_i .



Living cells are **NOT** at equilibrium;
they are in a **steady state**

Find $\Delta G'$ for ATP hydrolysis in a living *E. coli*, where
(ATP) \approx 8 mM, (ADP) \approx 1 mM, and (Pi) \approx 8 mM

$$\Delta G' = \Delta G^{\circ'} + RT \ln \frac{(\text{ADP})(\text{Pi})}{(\text{ATP})}$$

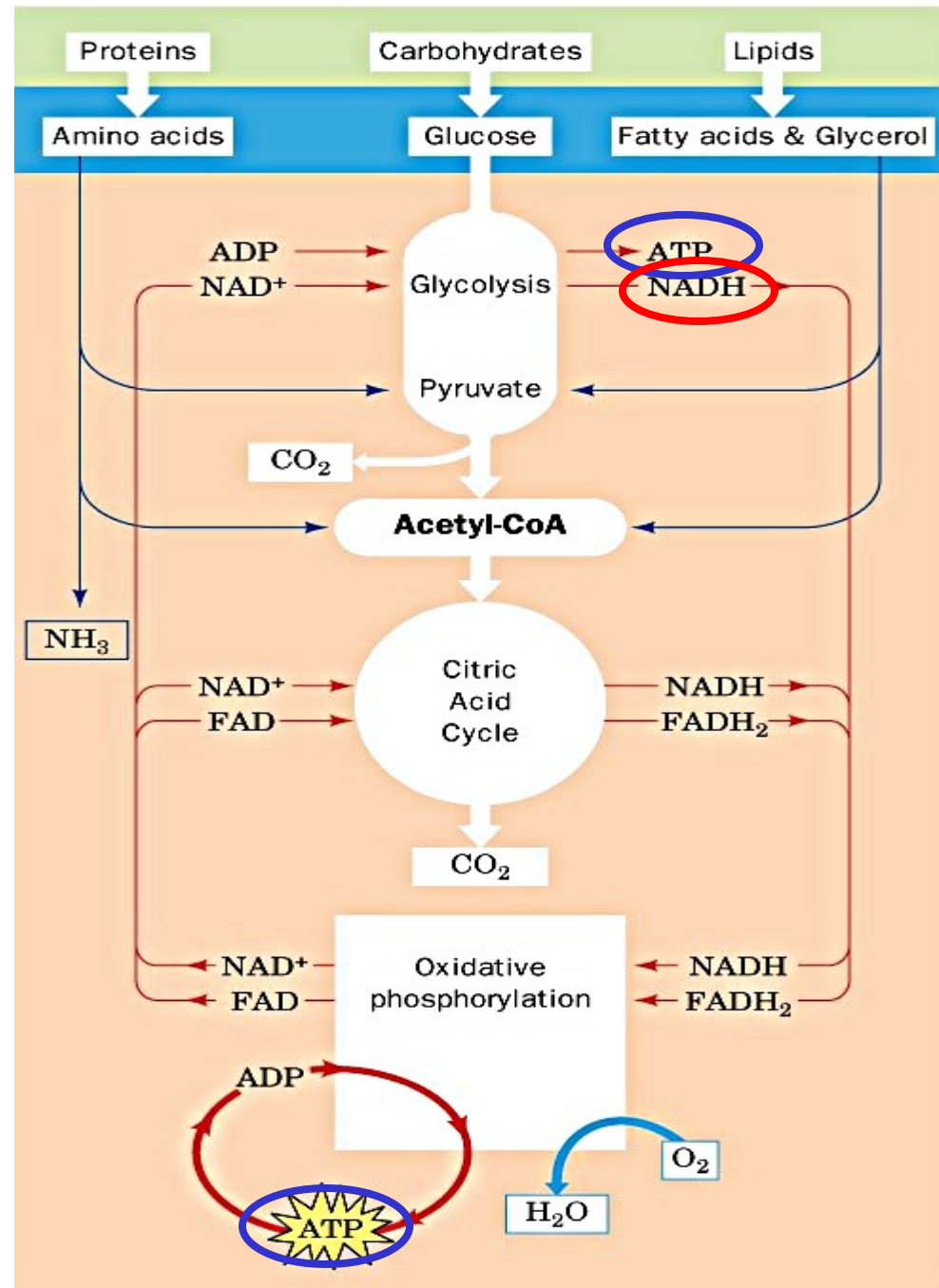
$$\begin{aligned} &= -31 \text{ KJ/mol} + 5.71 \log \left[\frac{(8 \times 10^{-3}) (1 \times 10^{-3})}{(8 \times 10^{-3})} \right] \\ &= -49 \text{ KJ/mol} \end{aligned}$$

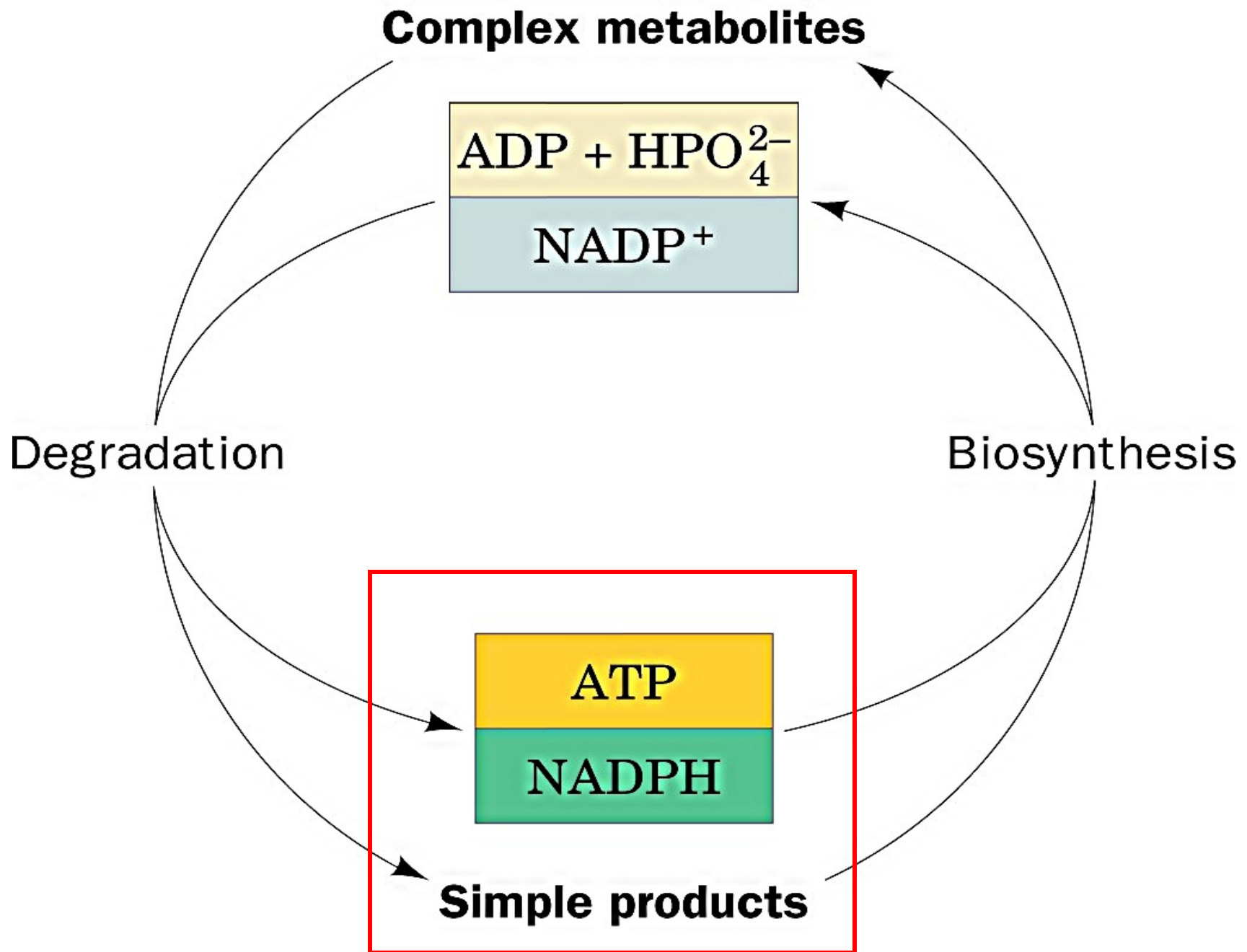
(Note: concentrations are MOLAR)

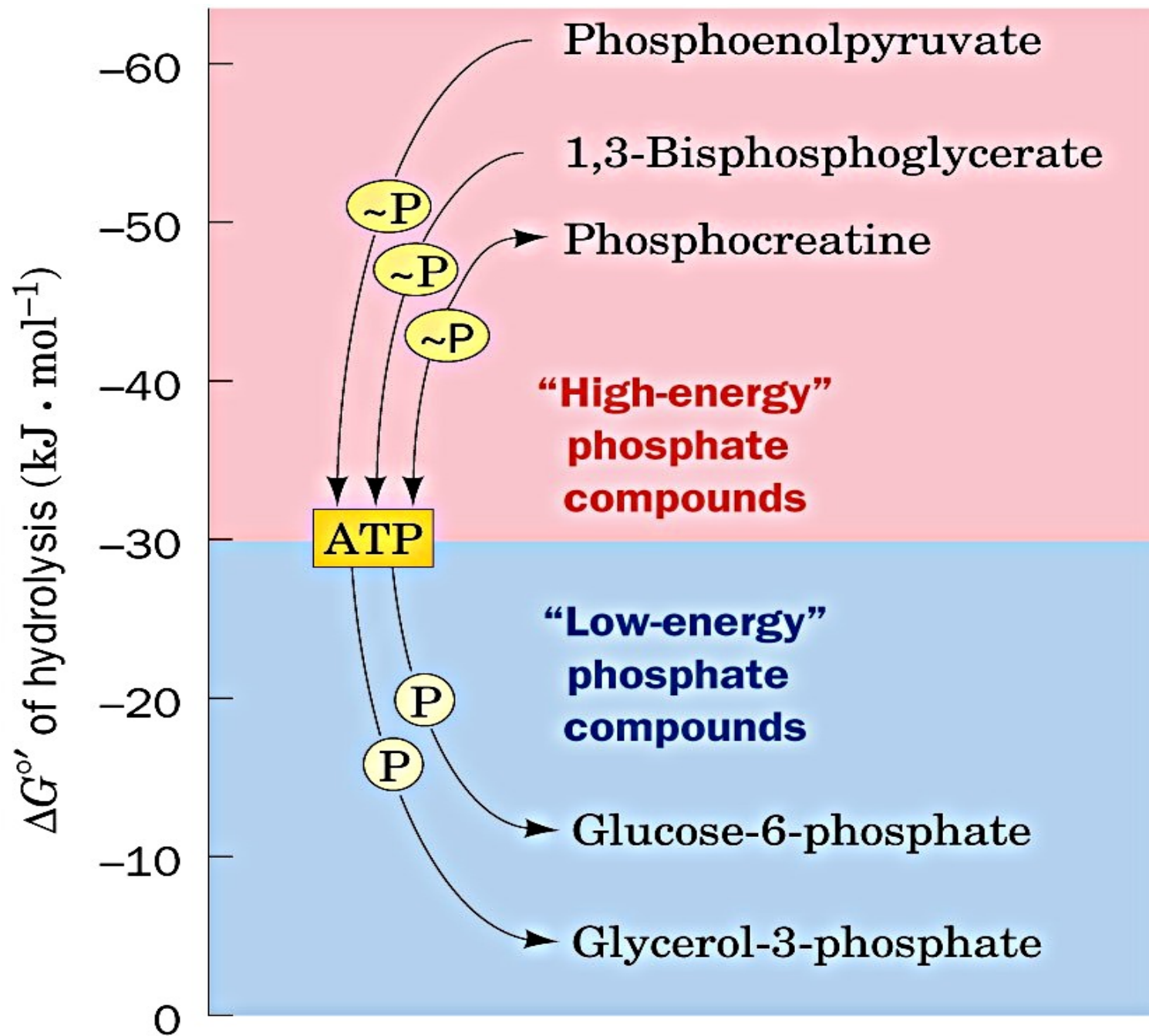
OR

$$\begin{aligned} &= -7.3 \text{ Kcal/mol} + 1.36 \log \left[\frac{(8 \times 10^{-3}) (1 \times 10^{-3})}{(8 \times 10^{-3})} \right] \\ &= -7.3 - 4.1 = -11.4 \text{ Kcal/mol} \end{aligned}$$

In Metabolism
- role of ATP
- role of NADH







Roles of ATP in Metabolism

Used for:

Activate nutrient breakdown

Biosynthetic reactions

Physiological processes

Formed by:

Substrate-level phosphorylation

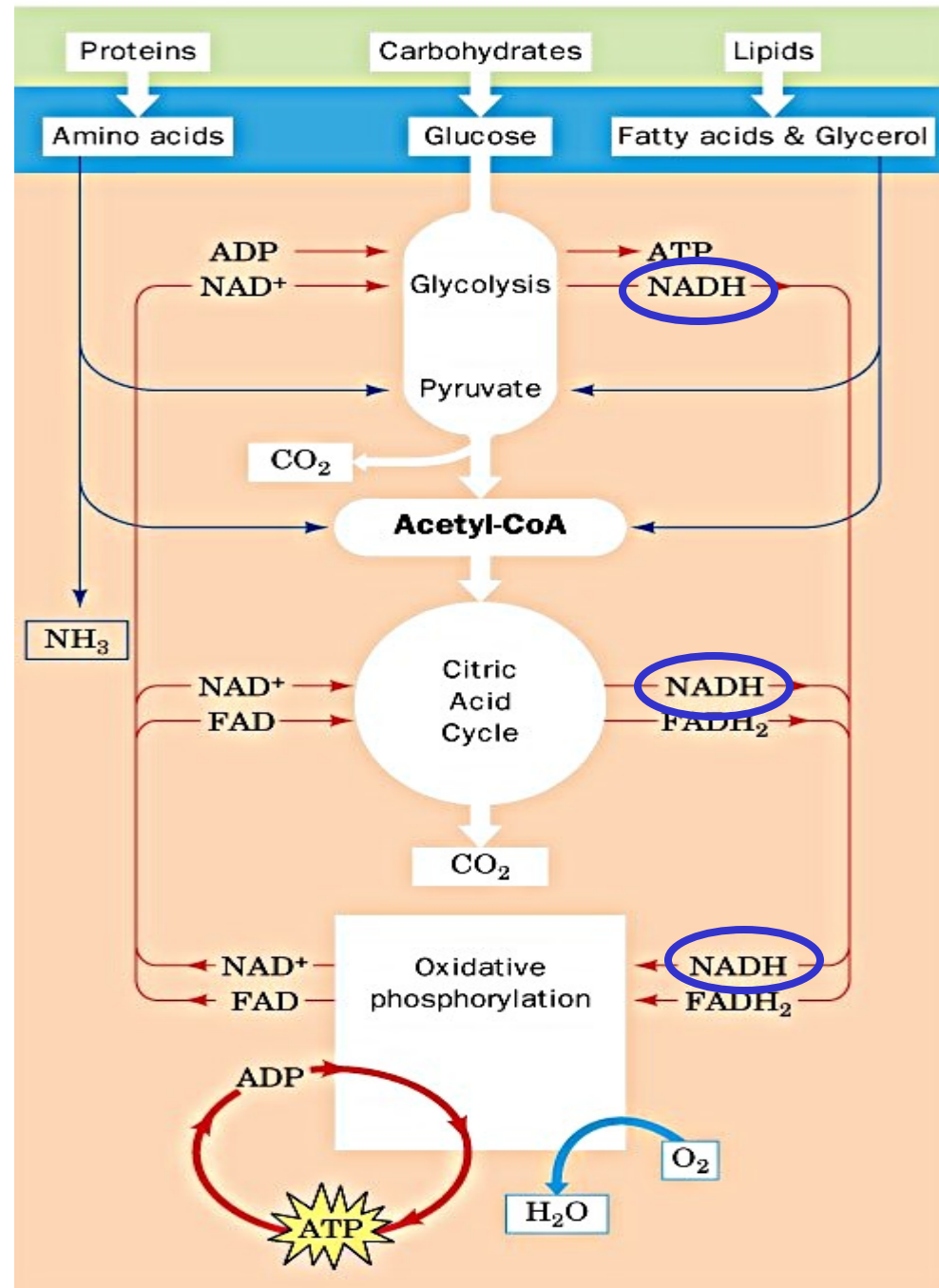
Oxidative phosphorylation / Photophosphorylation

Turnover - humans have about 50g ATP (0.09 moles); basal metabolism burns ~ 3 mol / hr; active metabolism is much higher.

Storage – Creatine Kinase (muscle, nerve cells)

In Metabolism

- role of NADH



Formal Oxidation States

Formal oxidation state (FOS) analysis is useful in examining redox reactions. Recall these rules from General Chem

1. C-C bonds are shared equally
2. More electronegative atoms take formal possession of the shared electrons
3. Sum of formal charges = charge on the molecule
4. Oxygen has FOS = -2; H has FOS = +1

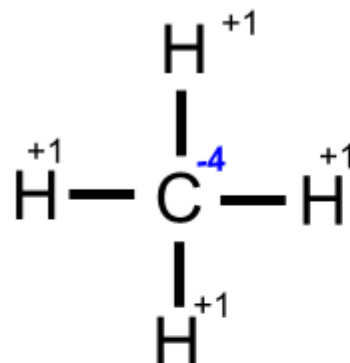
Formal Charge Examples

To find formal charge on central C of methane note that there are 4 Hs each with +1, and sum for this neutral molecule =0. Therefore formal charge on C must be -4, as shown in blue.

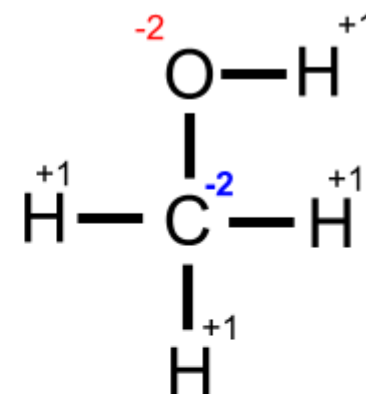
For methanol, central carbon must be -2 to balance fixed charges on O and Hs.

Note the charge on formic acid, +2, is the same as formate; the formal charges must sum to zero, or -1 respectively.

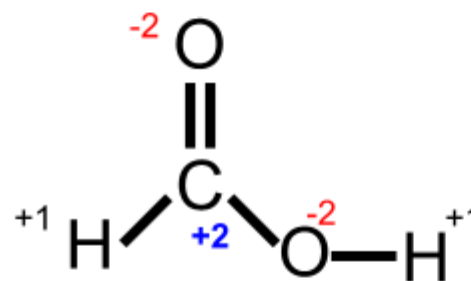
The acetaldehyde model reminds us that C-C bonds generate no formal charges, and the carbon dioxide molecule shows the wide range of formal charges on C. It can be any value between -4 and +4.



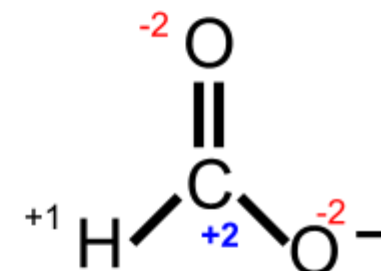
methane



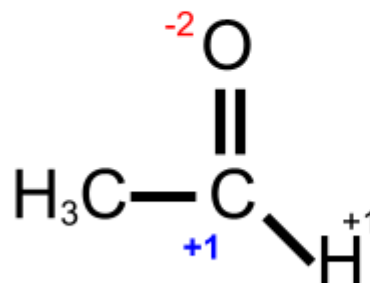
methanol



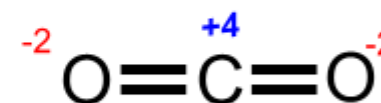
formic acid



formate

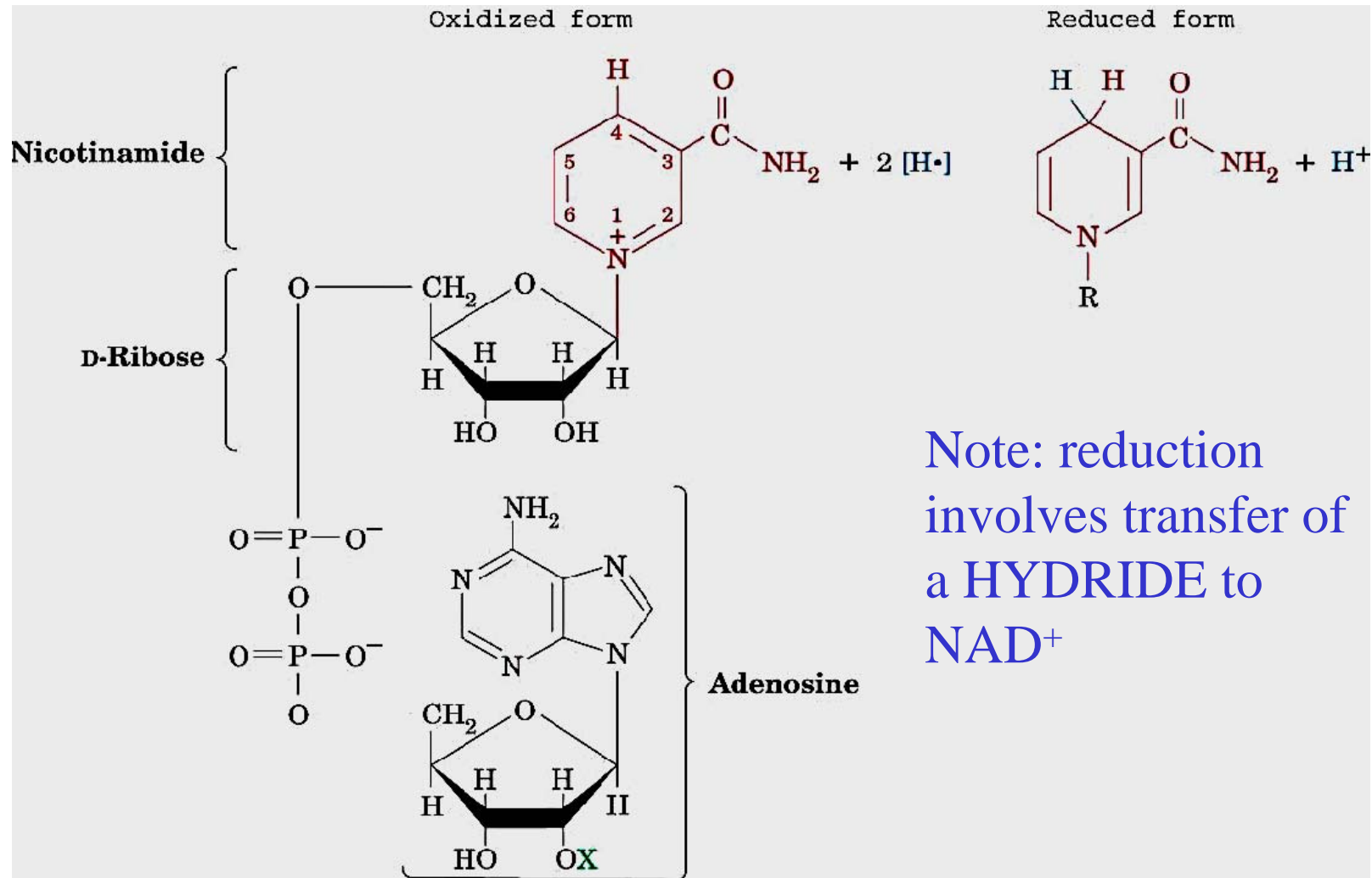


acetaldehyde



Carbon dioxide

Fig. 13-2 The molecular formula and **redox reactions** of the coenzyme nicotinamide adenine dinucleotide (NAD⁺).

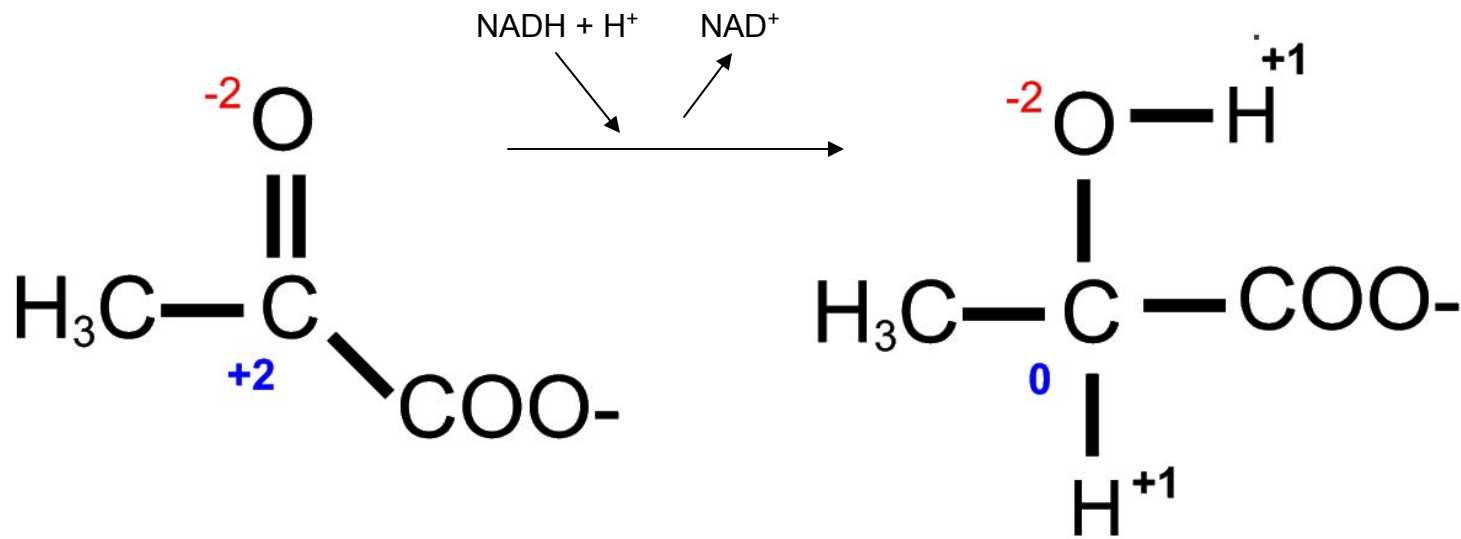


Note: reduction involves transfer of a HYDRIDE to NAD⁺

X = H Nicotinamide adenine dinucleotide (NAD⁺)
 X = PO₃²⁻ Nicotinamide adenine dinucleotide phosphate (NADP⁺)

A redox example

If $\text{CH}_3\text{-CO-COO}^-$ is reduced by NADH, to $\text{CH}_3\text{-CHOH-COO}^-$, the central carbon state goes from +2 to 0. It is reduced while NADH is oxidized to NAD^+



Redox Reactions

Oxidation of food stuffs releases energy. Some of the released energy is "converted" into high energy, reduced coenzymes such as NADH

Oxidation is a loss of electrons, hydrogen, or hydride, or the addition of oxygen.
Reduction is a gain of electrons, hydrogen, or hydride, or the loss of oxygen.

When something is **oxidized**, something else must be **reduced**.

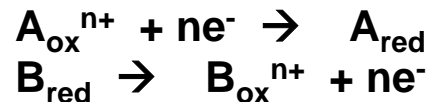


In this reaction, A is reduced (gains e^- s), and B is oxidized (loses e^- s).

A is the oxidizing agent, or oxidant

B is the reducing agent, or reductant

Break the overall reaction into two half-reactions:



The "propensity" to gain e^- s and become reduced is called the reduction potential; it can be measured as a half cell potential or voltage, and tabulated.

Standard Reduction Potentials

Half-Reaction	\mathcal{E}' (V)
$\frac{1}{2}\text{O}_2 + 2\text{H}^+ + 2e^- \rightleftharpoons \text{H}_2\text{O}$	0.815
Cytochrome a_3 (Fe^{3+}) + $e^- \rightleftharpoons$ cytochrome a_3 (Fe^{2+})	0.385
$\text{O}_2(\text{g}) + 2\text{H}^+ + 2e^- \rightleftharpoons \text{H}_2\text{O}_2$	0.295
Cytochrome a (Fe^{3+}) + $e^- \rightleftharpoons$ cytochrome a (Fe^{2+})	0.29
Cytochrome c (Fe^{3+}) + $e^- \rightleftharpoons$ cytochrome c (Fe^{2+})	0.235
Cytochrome b (Fe^{3+}) + $e^- \rightleftharpoons$ cytochrome b (Fe^{2+}) (<i>mitochondrial</i>)	0.077
Ubiquinone + $2\text{H}^+ + 2e^- \rightleftharpoons$ ubiquinol	0.045
Fumarate $^-$ + $2\text{H}^+ + 2e^- \rightleftharpoons$ succinate $^-$	0.031
FAD + $2\text{H}^+ + 2e^- \rightleftharpoons$ FADH $_2$ (<i>in flavoproteins</i>)	-0.040
Oxaloacetate $^-$ + $2\text{H}^+ + 2e^- \rightleftharpoons$ malate $^-$	-0.166
Pyruvate $^-$ + $2\text{H}^+ + 2e^- \rightleftharpoons$ lactate $^-$	-0.185
Acetaldehyde + $2\text{H}^+ + 2e^- \rightleftharpoons$ ethanol	-0.197
FAD + $2\text{H}^+ + 2e^- \rightleftharpoons$ FADH $_2$ (<i>free coenzyme</i>)	-0.219
Lipoic acid + $2\text{H}^+ + 2e^- \rightleftharpoons$ dihydrolipoic acid	-0.29
NAD $^+$ + $\text{H}^+ + 2e^- \rightleftharpoons$ NADH	-0.315
NADP $^+$ + $\text{H}^+ + 2e^- \rightleftharpoons$ NADPH	-0.320
Cystine + $2\text{H}^+ + 2e^- \rightleftharpoons$ 2 cysteine	-0.340
Acetoacetate $^-$ + $2\text{H}^+ + 2e^- \rightleftharpoons$ β -hydroxybutyrate	-0.346
$\text{H}^+ + e^- \rightleftharpoons \frac{1}{2}\text{H}_2$	-0.421
Acetate $^-$ + $3\text{H}^+ + 2e^- \rightleftharpoons$ acetaldehyde + H_2O	-0.581

Reduction potential depends on concentration: the Nernst Equation

Here \mathcal{E} is redox potential, not the internal energy.

$$\mathcal{E}' = \mathcal{E}'^0 + (RT/nF)\ln(\text{ox/red}) = \mathcal{E}'^0 - (RT/nF)\ln(\text{red/ox})$$

Substituting for R, T, F, and converting to base 10 logs, gives:

$$\mathcal{E}' = \mathcal{E}'^0 + (0.06/n)\log(\text{ox/red}).$$

For example, a system where FAD=10 mM and FADH₂ = 1 mM

$$\mathcal{E}' = -0.219 + (0.06/2)\log(0.01/0.001) = -0.219 + 0.03 = -0.189 .$$

The energetics of redox systems can be converted to more traditional free energy as: $\Delta\mathbf{G} = -n\mathbf{F}\mathcal{E}$

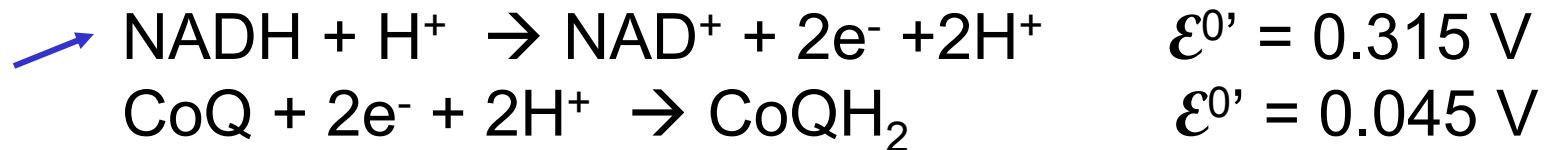
Note a system is spontaneous when $\Delta\mathbf{G}$ is negative or \mathcal{E} is positive.

A worked example

In the first stage of the ETS, electrons from NADH are passed through complex 1 to CoQ. NADH is oxidized and CoQ reduced.

The overall reaction, and subsequent voltage drop is:

Note:
written as
oxidation.



In terms of more conventional free energy measures:

$$\Delta G^{0'} = -nF\mathcal{E}^{0'} = -2 \times 96 \text{ KJ/molV} \times 0.36 \text{ V} = -69.5 \text{ KJ/mol}$$

$$\begin{aligned} \text{Or: } \Delta G^{0'} &= -nF\mathcal{E}^{0'} = -2 \times 23 \text{ Kcal/molV} \times 0.36 \text{ V} \\ &= -16.6 \text{ Kcal/mol} \end{aligned}$$

Thermo Home Work (Sorry)

- Problems from Voet & Voet 3rd Ed
- I don't care if you work alone or communally or what, but I strongly suggest you practice these thermo problems.
- Chapter 3: 2, 5, 8, 10b, 11
- Chapter 16: 6, 11
- Turn in for credit, and be aware problems related to this WILL be on the next test.