

L2: Bioenergetics

Dr. P. Satpati,
BSBE, IIT Guwahati



Quantitative answers for the following questions

1. Will this happen spontaneously ? (Driving Force)
2. **How much will this happen ?**
3. **Relation between 1 and 2 ?**
4. How all this is related to biology

Biological Thermodynamics

$$\Delta G = \Delta H - T\Delta S$$

The **Enthalpic** term

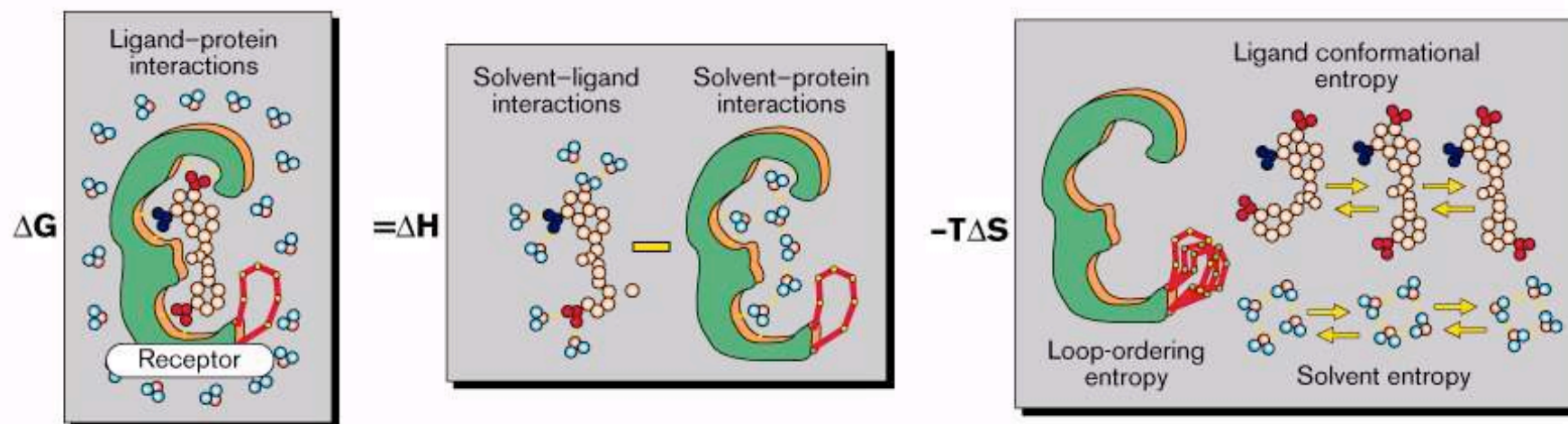
- Changes in bonding
 - van der Waals
 - Hydrogen bonding
 - Charge interactions

The **Entropic** term

- Changes the arrangement of the solvent or counterions
- Reflects the degrees of freedom
- Rotational & Translational changes

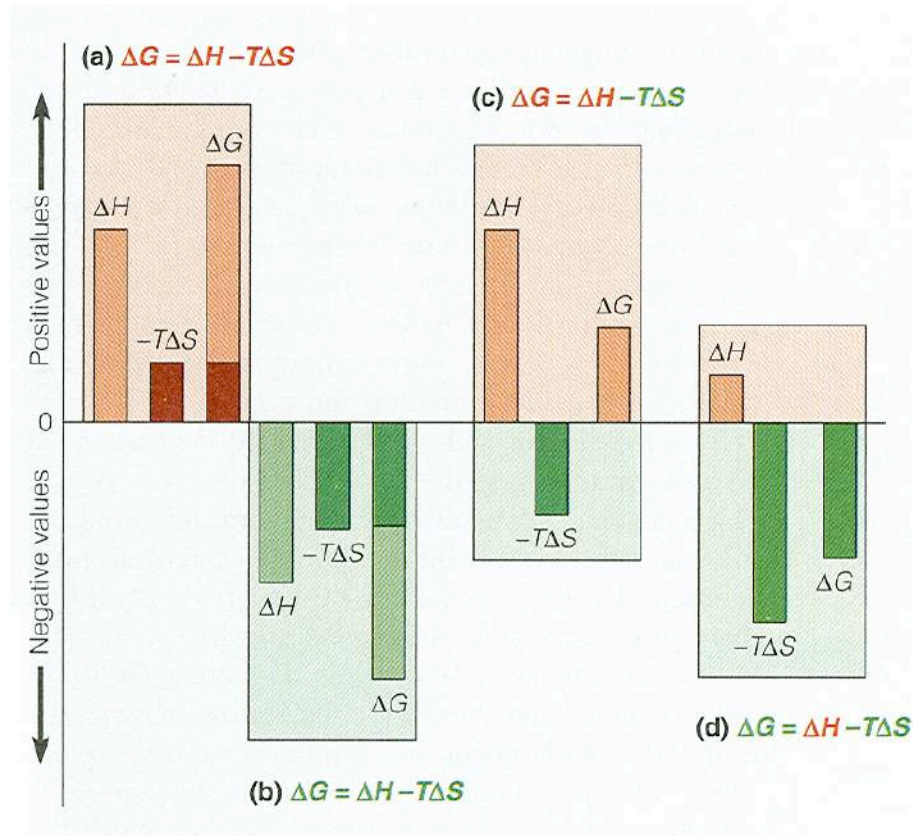
Biological Thermodynamics

$$\Delta G = \Delta H - T\Delta S$$



Biological Thermodynamics

The Gibbs free energy (ΔG)

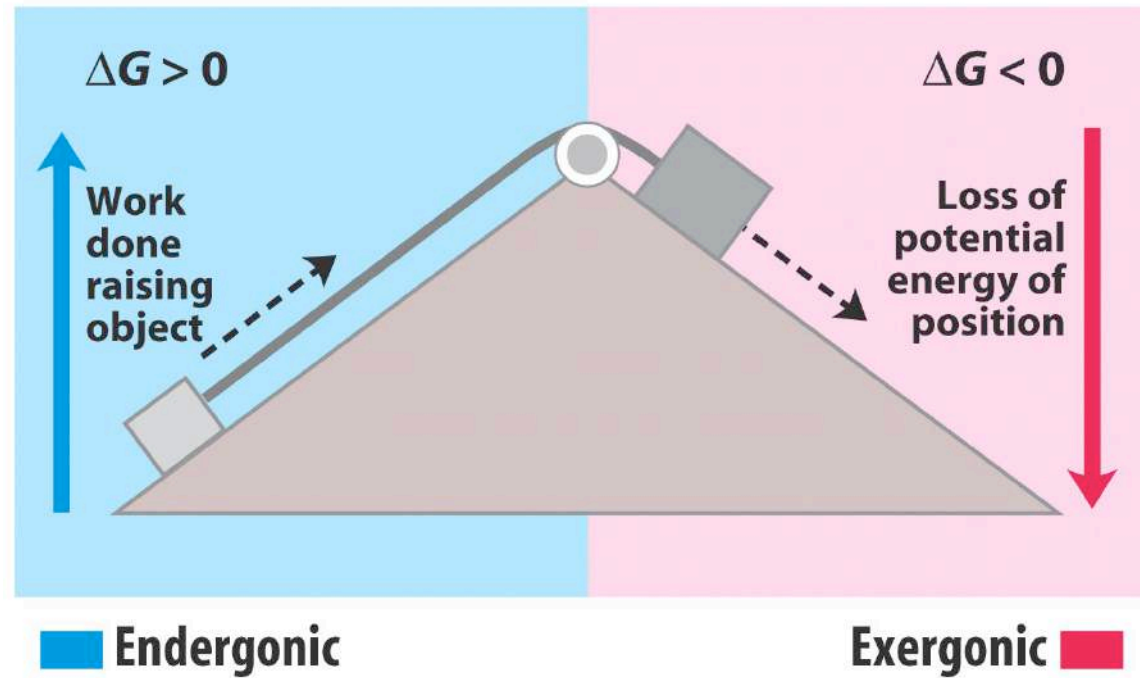


If ΔG is ...	The process is ...
Negative	Thermodynamically favored
Zero	Reversible; at equilibrium
Positive	Thermodynamically unfavored; reverse process is favored

ΔH	ΔS	Low T	High T
+	+	ΔG positive; not favored	ΔG negative; favored
+	-	ΔG positive; not favored	ΔG positive; not favored
-	+	ΔG negative; favored	ΔG negative; favored
-	-	ΔG negative; favored	ΔG positive; not favored

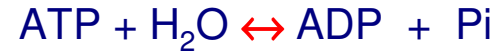
Biological Thermodynamics

The Gibbs free energy (ΔG)



Energy coupling links reactions

Energy Coupling:



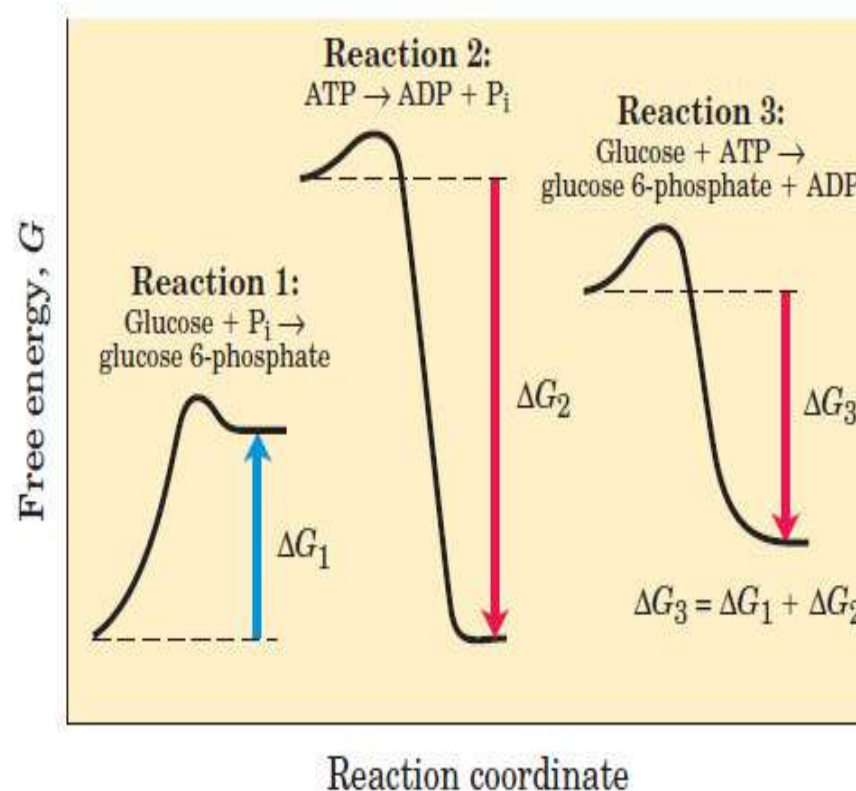
$$\Delta G^0 = -30.5 \text{ kJ/mol}$$



$$\Delta G^0 = +14 \text{ kJ/mol}$$

Half-reactions

Coupled reaction:



Energy Coupling: By common intermediate

Reaction 1:



Reaction 2:



Overall spontaneous reaction:



Pyrophosphate (PPi) is often the product of a reaction that needs a driving force.

Its spontaneous hydrolysis, catalyzed by Pyrophosphatase enzyme, drives the reaction for which PPi is a product.

What we learned so far

- Criteria for deciding whether a reaction will be spontaneous (Driving force of a process).
- Biological system can couple reaction free energies for function

Missing points

- Chemical processes are not “all or nothing”. An equilibrium is reached where both reactant and product is present.
- **Missing link : Free energy change and concentration of reactant and product.**

Quantitative relationship : Free energy change and concentration of reactant and product.

Plan of action: Establish the relationship for a simple case of ideal gases and by analogy for reactions in liquid

Quantitative relationship : Free energy change and concentration of reactant and product.

$$G = H - TS$$

$$\Rightarrow dG = dH - TdS - SdT$$

$$\Rightarrow dG = dH - TdS - SdT$$

By definition $H = U + PV$?

$$\Rightarrow dG = dU + PdV + VdP - TdS - SdT$$

1st Law $dU = Q + W = TdS - PdV$ (For reversible process, No Non-Mech work)

$$\Rightarrow dG = TdS - PdV + PdV + VdP - TdS - SdT$$

$$\Rightarrow dG = VdP - SdT$$

At constant Temp

$$\Rightarrow dG = VdP$$

For 1 mole ideal gas, $PV = RT$

$$\Rightarrow dG = \frac{RT}{P} dP$$

$$\Rightarrow dG = \frac{RT}{P} dP$$

Integrate from $P^0=1 \text{ atm}$ to P

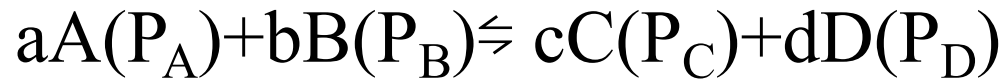
$$\Rightarrow G - G^0 = RT \ln(P/P^0)$$

Lets return to a prototype reaction : $aA(P_A)+bB(P_B) \rightleftharpoons cC(P_C)+dD(P_D)$

$$\Delta G = (G_{product} - G_{reactant}) = c G_C + d G_D - a G_A - b G_B$$

Substituting $G - G^0 = RT \ln(P/P^0)$

$$\Rightarrow \Delta G = (c G_C^0 + d G_D^0 - a G_A^0 - b G_B^0) + RT \ln \left[\frac{\left(\frac{P_C}{P^0}\right)^c \left(\frac{P_D}{P^0}\right)^d}{\left(\frac{P_A}{P^0}\right)^a \left(\frac{P_B}{P^0}\right)^b} \right]$$



$$\Rightarrow \Delta G = (c G^0_C + d G^0_D - a G^0_A - b G^0_B) + RT \ln \left[\frac{\left(\frac{P_C}{P^0}\right)^c \left(\frac{P_D}{P^0}\right)^d}{\left(\frac{P_A}{P^0}\right)^a \left(\frac{P_B}{P^0}\right)^b} \right]$$

$$\Rightarrow \Delta G = \Delta G^0 + RT \ln \left[\frac{\left(\frac{P_C}{P^0}\right)^c \left(\frac{P_D}{P^0}\right)^d}{\left(\frac{P_A}{P^0}\right)^a \left(\frac{P_B}{P^0}\right)^b} \right]$$

At equilibrium

$$\Rightarrow 0 = \Delta G^0 + RT \ln \left[\frac{\left(\frac{P_C}{P^0}\right)^c \left(\frac{P_D}{P^0}\right)^d}{\left(\frac{P_A}{P^0}\right)^a \left(\frac{P_B}{P^0}\right)^b} \right]_{\text{eqm}}$$

$$\Rightarrow \Delta G^0 = - RT \ln \left[\frac{\left(\frac{P_C}{P^0}\right)^c \left(\frac{P_D}{P^0}\right)^d}{\left(\frac{P_A}{P^0}\right)^a \left(\frac{P_B}{P^0}\right)^b} \right]_{\text{eqm}}$$

$$\Rightarrow \Delta G^0 = - RT \ln K_{\text{eqm}}$$

ΔG^0 = Standard free energy change is a constant (fixed T, P) representing a hypothetical reaction with all of reactants and products at a pressure of 1 atm.

K_{eqm} = Equilibrium constant

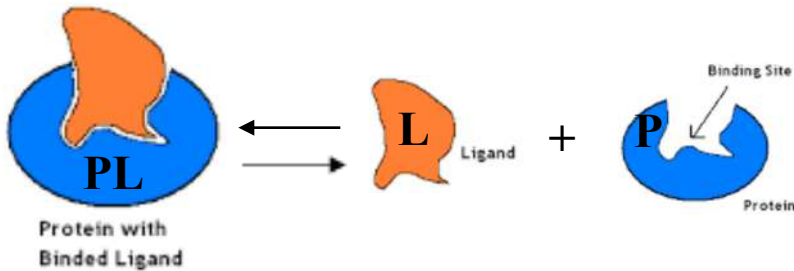
Biological reactions do not occur in the gas phase. What about the free energy in solution. Conceptually no difference. Replace pressure by concentrations.

$$G - G^0 = RT \ln(\text{conc}/\text{conc}^0)$$

$$\Rightarrow \Delta G^0 = -RT \ln \left[\frac{\left(\frac{C_C}{C^0}\right)^c \left(\frac{C_D}{C^0}\right)^d}{\left(\frac{C_A}{C^0}\right)^a \left(\frac{C_B}{C^0}\right)^b} \right]_{\text{eqm}} = -RT \ln K_{\text{eqm}}$$

LIGAND BINDING:

Equation describing **dissociation** of the ligand (L) from the protein (P)



$$K_d = \frac{[P][L]}{[PL]}$$

- The concentrations of free protein [P], free ligand [L], and the P•L complex [PL] in this expression are the equilibrium concentrations. **units of K_d for this protein•ligand dissociation reaction?**
- Suppose a protein can bind either of 2 different ligands. K_d is 0.1 μM for one ligand (A), and 1.0 μM for the other ligand (B). **Which ligand shows *higher affinity binding* (tighter binding, a stronger tendency to associate), ligand A or ligand B?**

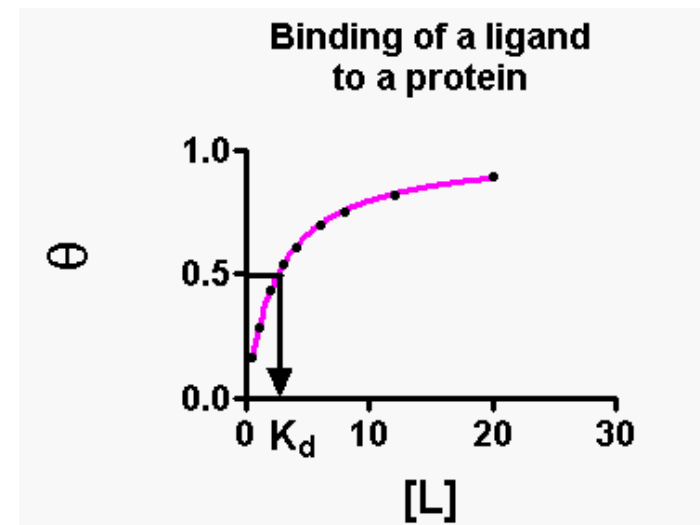
fraction of the total binding sites (θ , **unit less**) on the protein $[P]_{\text{total}}$

$$\theta = \frac{[L]_{\text{bound}}}{[P]_{\text{total}}} = \frac{[PL]}{[P] + [PL]}$$

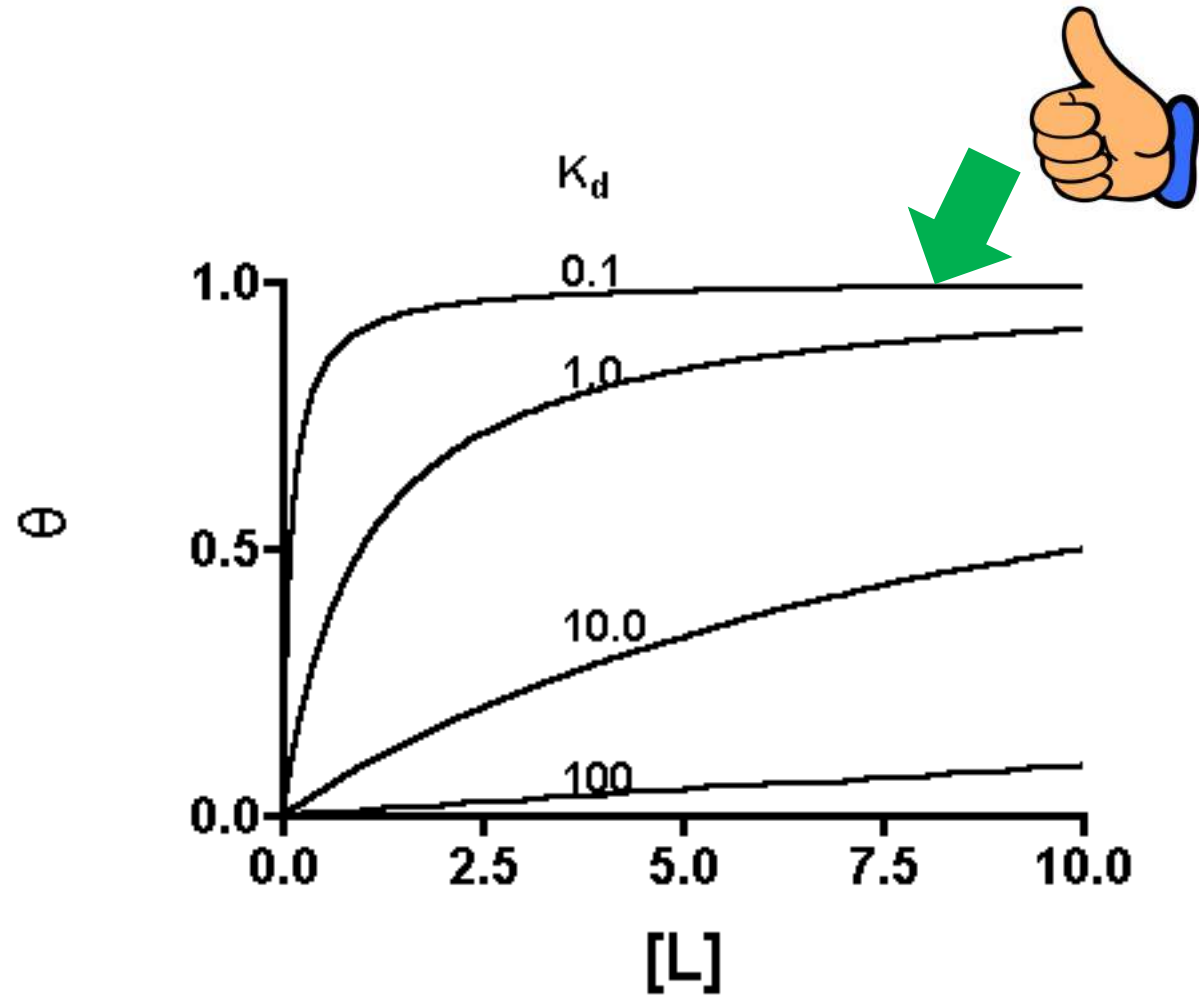
Combining expressions for K_d and θ

$$\theta = \frac{[L]}{K_d + [L]}$$

NOTE: K_d is the *concentration of ligand needed to HALF-SATURATE* the binding sites.



Which plot is for the best inhibitor ?



Thermodynamic
equilibrium
constant

Standard
entropy change
of reaction

Standard
enthalpy change
of reaction

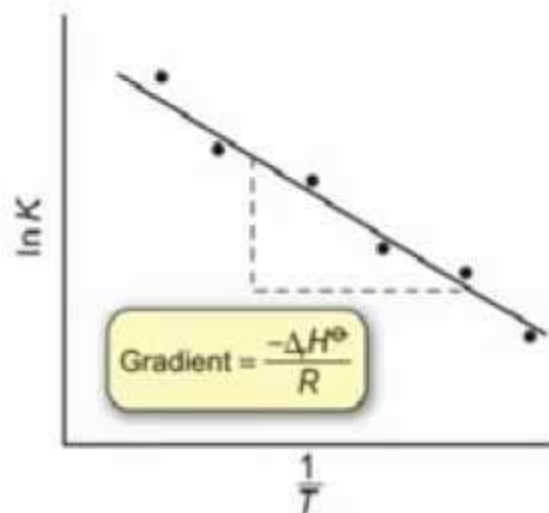
$$\ln K = \frac{\Delta_r S^\ominus}{R} - \frac{\Delta_r H^\ominus}{RT} \quad (15.10)$$

Gas constant
 $R = 8.314 \text{ J K}^{-1} \text{ mol}^{-1}$

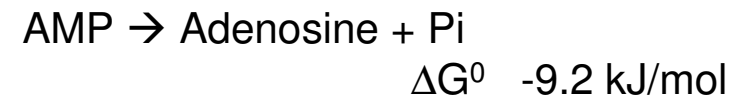
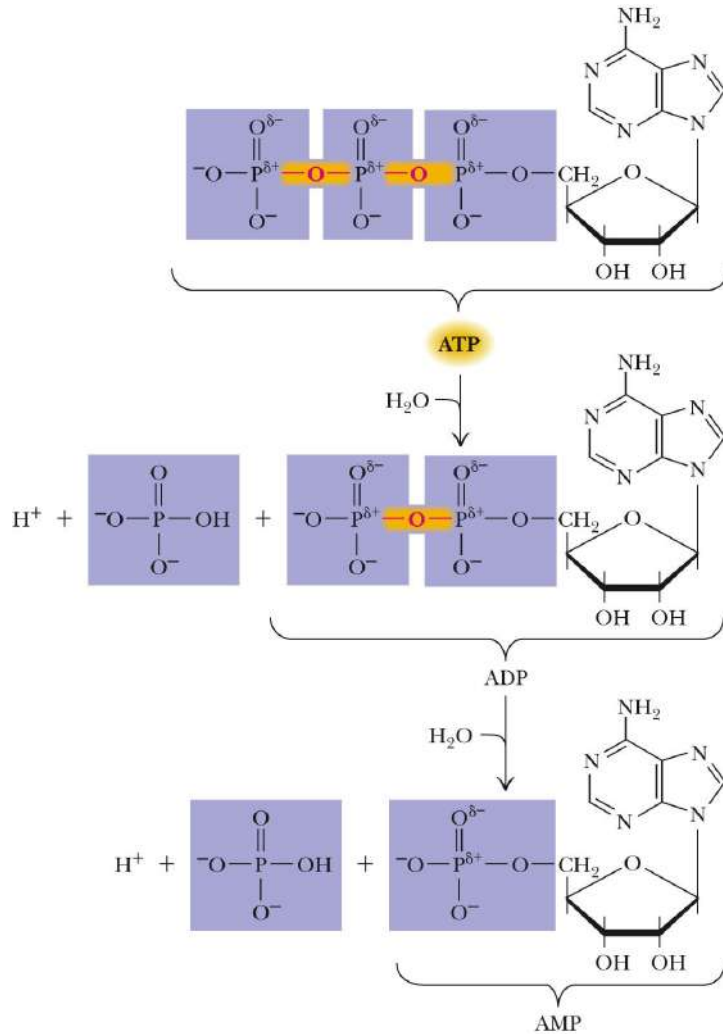
Temperature
(in kelvin)

$$\Delta_r G^\ominus = -RT \ln K \quad (15.4)$$

$$\Delta_r G^\ominus = \Delta_r H^\ominus - T \Delta_r S^\ominus \quad (14.16)$$



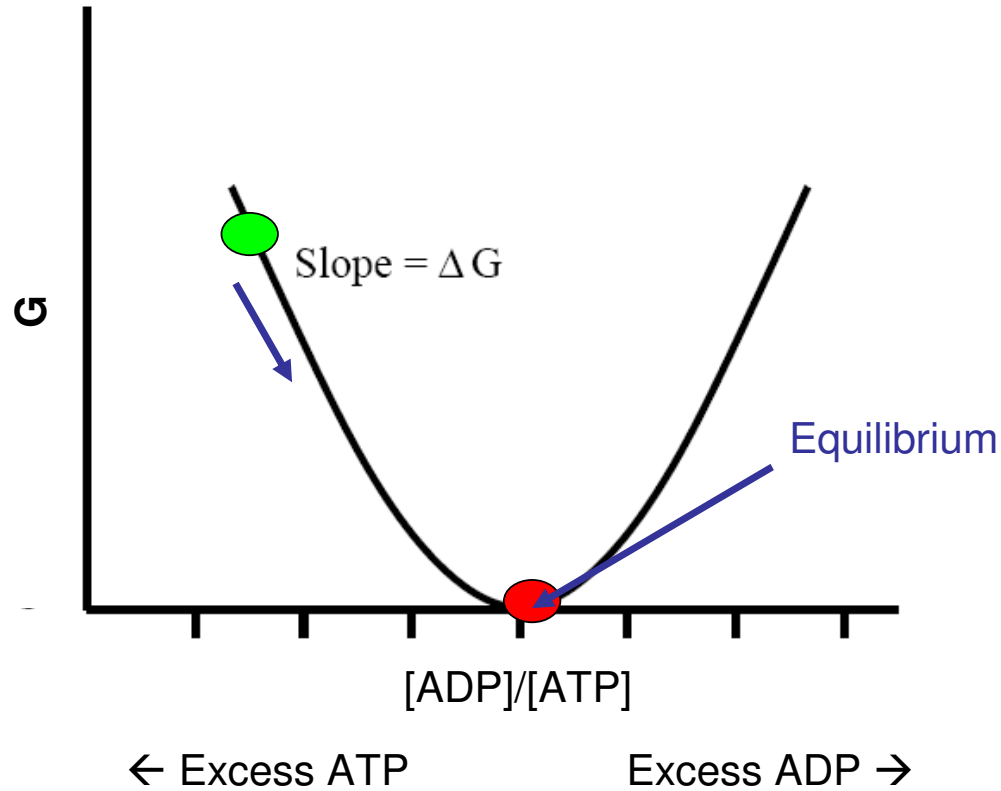
Energetics of ATP



Factors in the large negative ΔG^0 :

- electrostatic repulsion.
- stabilization of products by resonance.

System Away from Equilibrium



For ATP hydrolysis to ADP

$$K'_{eq} = 2 \times 10^5 \text{ M},$$

For constant $[P_i] = 0.01 \text{ M}$

$$[ADP]_{eq}/[ATP]_{eq} = 2 \times 10^5 / 0.01 \\ = 2 \times 10^7 \text{ M}$$



In the example in last slide:

$$[ADP]/[ATP] = 10^{-3}$$



Displacement from equilibrium gives the cell its ability to do work!

Energetics of ATP



Concentration of cellular P_i (remains almost constant), $[\text{P}_i] = 10^{-2} \text{ M}$

Concentration of ATP, $[\text{ATP}] = 10^{-2} \text{ M}$

Concentration of ADP, $[\text{ADP}] = 10^{-5} \text{ M}$

$$R = 8.3 \text{ J.mol}^{-1}.\text{K}^{-1}; T = 310 \text{ K}$$

$$\begin{aligned} \Delta G &= \Delta G^0 + RT \ln ([\text{ADP}][\text{P}_i]/[\text{ATP}]) \\ &= -30.5 + 8.3 \cdot 310 \ln(10^{-5}) = -60 \text{ kJ/mol} \end{aligned}$$



Quantitative answers for the following questions

1. Will this happen spontaneously ? (Driving Force)

2. **How much will this happen ?**

3. **Relation between 1 and 2 ?**

4. **How all this is related to biology**

2. Until $\Delta G = 0$
3. $\Delta G^0 = -RT \ln K_{eqm}$
4. Coupling of different reaction.

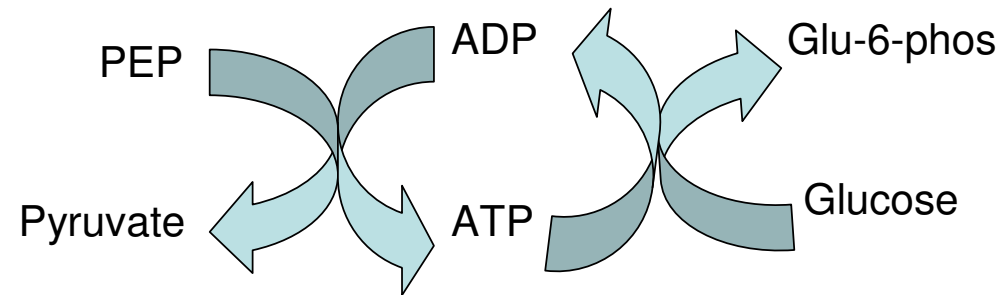
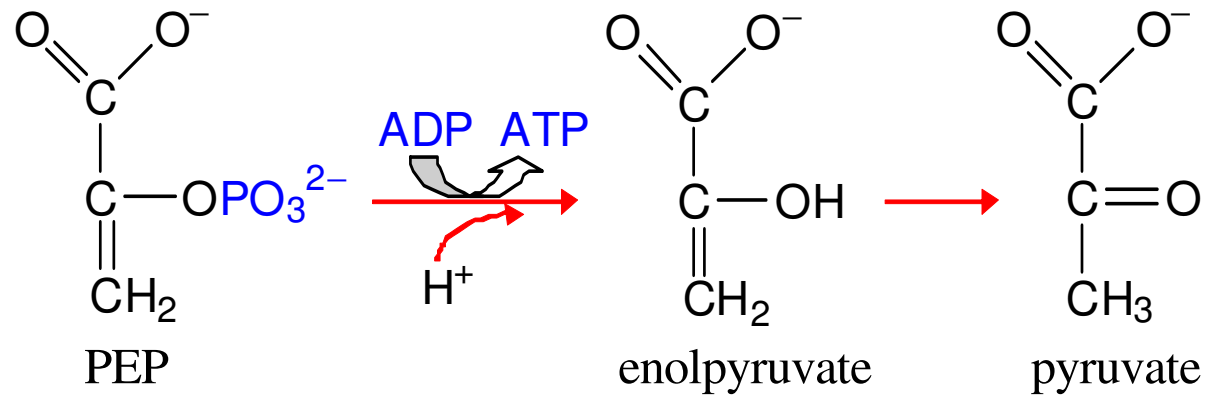
Other High Energy Molecules

Compound	ΔG° of phosphate hydrolysis, kJ/mol
Phosphoenolpyruvate (PEP)	– 61.9
Phosphocreatine	– 43.1
Pyrophosphate	– 33.5
ATP (to ADP)	– 30.5
Glucose-6-phosphate	– 13.8
Glycerol-3-phosphate	– 9.2

ATP is **intermediate** among examples.

ATP can thus act as a P_i donor, & ATP can be synthesized by P_i transfer, e.g., from PEP.

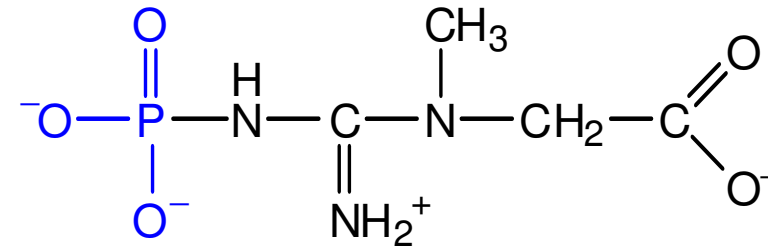
Coupling in series



Many of this type of coupled reactions involve multi-enzyme complex

Other High Energy Molecules

Phosphocreatine a "high energy" phosphate linkage, is used in nerve & muscle for **storage of ~P bonds**.



phosphocreatine

Creatine Kinase catalyzes:



- Phosphocreatine is produced when ATP levels are high.
- When ATP is depleted during **exercise** in muscle, phosphate is transferred from phosphocreatine to ADP, to **replenish ATP**.

Energy Coupling: By common intermediate

Reaction 1:



Reaction 2:



Overall spontaneous reaction:



Pyrophosphate (PPi) is often the product of a reaction that needs a driving force.

Its spontaneous hydrolysis, catalyzed by Pyrophosphatase enzyme, drives the reaction for which PPi is a product.

Reaction 1: $\text{ATP} + \text{H}_2\text{O} \leftrightarrow \text{ADP} + \text{Pi}$

$$K'_{\text{eq1}} = [\text{ADP}][\text{Pi}]/[\text{ATP}] \quad (\text{note water not considered})$$
$$= 2 \times 10^5 \text{ M}$$

Reaction 2: $\text{Pi} + \text{glucose} \leftrightarrow \text{glucose-6-P} + \text{H}_2\text{O}$

$$K'_{\text{eq2}} = [\text{glucose-6-phosphate}]/[\text{glucose}][\text{Pi}]$$
$$= 3.9 \times 10^{-3} \text{ M}^{-1}$$

So for the coupled reaction,

$$K'_{\text{eq}} = K'_{\text{eq1}} \times K'_{\text{eq2}} = 7.8 \times 10^2$$