Physical Principles in Biology
Biology 3550
Spring 2025

Lecture 29

Enzymatic Coupling and Introduction to Water

Wednesday, 26 March

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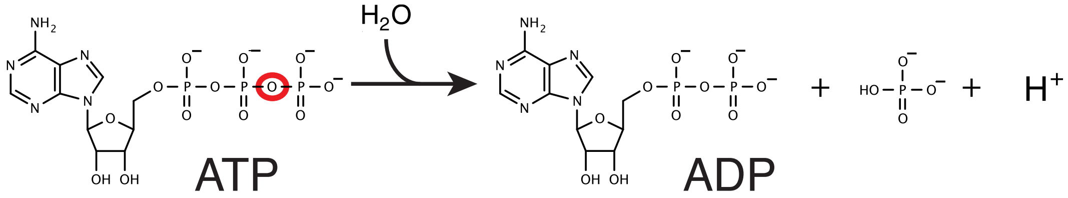
Announcements

- Quiz 4:
  - Friday, 28 March
  - 25 min, second half of class
  - Will cover material on thermodynamics
- Problem set 4
  - Due Friday, 28 March, 11:59 PM

Another “High-energy” Reaction



- $\Delta G^\circ = -30\,\mathrm{kJ/mol}$

Clicker Question #1

What is the equilibrium constant for the reaction?

$$\text{ATP} \rightleftharpoons \text{ADP} + \text{P}_\text{i} \qquad \Delta G^\circ = -30\,\text{kJ/mol}$$

A) $\approx 10^{-5}$ M

B) $\approx 10^{-3}$ M

C) ≈ 10 M

D) $\approx 10^{3}$ M

E) $\approx 10^{5}$ M

$$R = 8.314\,\text{J/(K}\cdot\text{mol)} \qquad T = 298\,\text{K}$$

Calculation of Equilibrium Constant from ΔG°

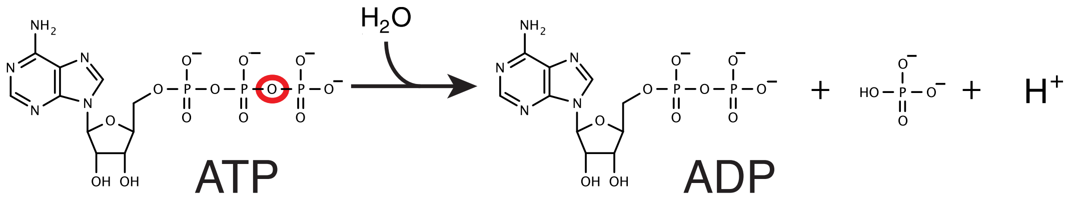
$$\Delta G^\circ = -30\,\mathrm{kJ/mol} \qquad R = 8.314\,\mathrm{J/(K\cdot mol)} \qquad T = 298\,\mathrm{K}$$

$$\Delta G^\circ = -RT\ln K_{\mathrm{eq}}$$
$$\ln K_{\mathrm{eq}} = -\Delta G^\circ/(RT)$$
$$K_{\mathrm{eq}} = e^{-\Delta G^\circ/(RT)}$$

$$-\Delta G^\circ/(RT) = 30\,\mathrm{kJ/mol} \div \big(8.314\,\mathrm{J/(K\cdot mol)}\cdot 298\,\mathrm{K}\big)$$
$$= 30\times 10^3\,\mathrm{J/mol} \div 2.48\times 10^3\,\mathrm{J/mol} = 12$$

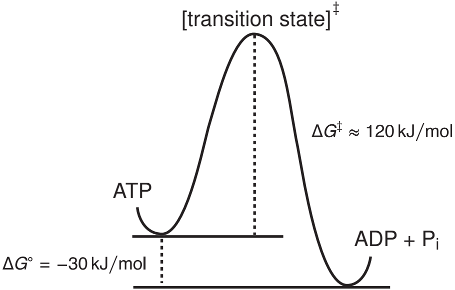
$$K_{\mathrm{eq}} = e^{12} = 1.8\times 10^5\,\mathrm{M}$$

ATP Hydrolysis



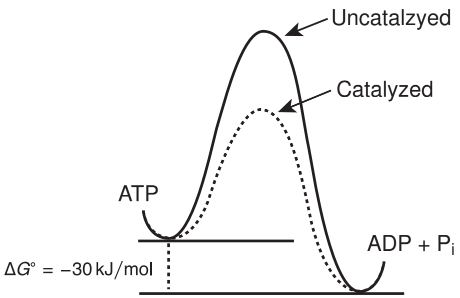
- $\Delta G^\circ = -30\,\text{kJ/mol}$, $K_{eq} \approx 2 \times 10^5$ M
- Why is the reaction so favorable?
  - High density of negative phosphate charges is reduced.
  - More resonance stabilization in ADP and P_i.
  - More favorable interaction with water by ADP and P_i.

Kinetics of ATP Hydrolysis



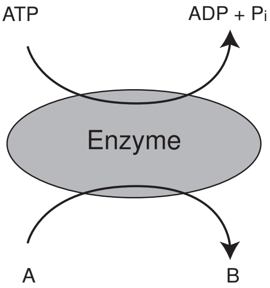
- Half-time is $\approx$ 20 days at neutral pH and 60°C.
- Transition state is a high energy state with equal probability of breaking down in either direction.
- Reaction rate is proportional to probability of forming the transition state.
- See the lobby of the Henry Eyring Building!

Catalysis of ATP Hydrolysis



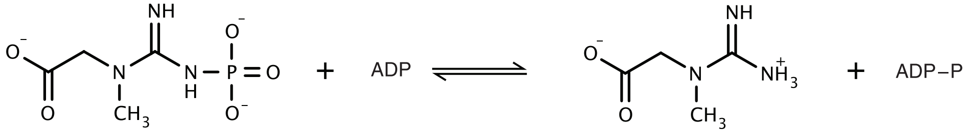
- Enzymes catalyze chemical reactions by lowering transition-state energy.
- Enzymes create micro-environments that favor forming the transition state.
- Simply catalyzing ATP hydrolysis is not useful!

Enzymatic Coupling



- Enzyme mechanistically couples reactions.
- ATP $\longrightarrow$ ADP $+$ P_i can't occur without A $\longrightarrow$ B
- A $\longrightarrow$ B can't occur without ATP $\longrightarrow$ ADP $+$ P_i
- Coupled "reaction" can be physical motion or transport across membranes.

Enzymatic Coupling: Creatine Kinase



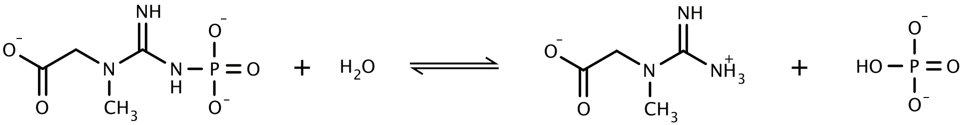
Creatine phosphate (CP)

Creatine (C)

- Coupled reactions

$$
\begin{aligned}
\mathrm{CP} &\rightleftharpoons \mathrm{C} + \mathrm{P_i} \\
\mathrm{ADP} + \mathrm{P_i} &\rightleftharpoons \mathrm{ATP} \\
\hline
\mathrm{ADP} + \mathrm{CP} &\rightleftharpoons \mathrm{ATP} + \mathrm{C}
\end{aligned}
$$

Hydrolysis of Creatine Phosphate

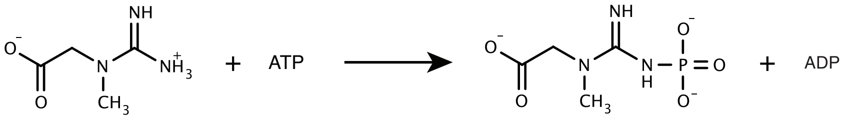


$\Delta G^\circ = -43\,\mathrm{kJ/mol}$; more favorable than ATP hydrolysis.

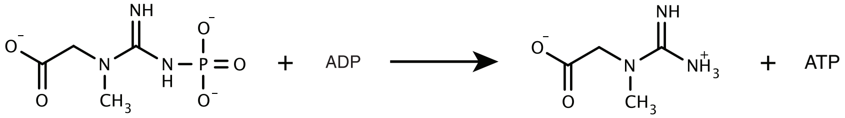
Clicker Question #2

In which direction is the reaction favorable under standard-state conditions?

A)



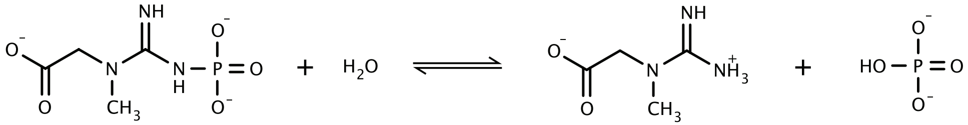
B)



C) Neither direction is more favorable than the other.

Enzymatic Coupling: Creatine Kinase

- Hydrolysis of creatine phosphate:



$\Delta G^\circ = -43\,\mathrm{kJ/mol}$; more favorable than ATP hydrolysis.

- Coupled reactions

$$\mathrm{ADP} + \mathrm{P_i} \rightleftharpoons \mathrm{ATP} \qquad \Delta G^\circ = 30\,\mathrm{kJ/mol}$$
$$\mathrm{CP} \rightleftharpoons \mathrm{C} + \mathrm{P_i} \qquad \Delta G^\circ = -43\,\mathrm{kJ/mol}$$

$$\mathrm{ADP} + \mathrm{CP} \rightleftharpoons \mathrm{ATP} + \mathrm{C} \qquad \Delta G^\circ = -13\,\mathrm{kJ/mol}$$

Phosphorylation of ADP by creatine phosphate is favored.

(at standard-state concentrations!)

Creatine Kinase in Muscle Cells

$$\text{ADP} + \text{CP} \rightleftharpoons \text{ATP} + \text{C} \qquad \Delta G^\circ = -13\,\text{kJ/mol}$$

- Typical concentrations in resting muscle cells:
  4 mM ATP
  0.013 mM ADP
  25 mM creatine phosphate
  13 mM creatine
- Calculate ΔG for these concentrations:

$$\Delta G = \Delta G^\circ + RT \ln \frac{[\text{ATP}][\text{C}]}{[\text{ADP}][\text{CP}]}$$

$$= -13\,\text{kJ/mol} + RT \ln \frac{0.004\,\text{M} \cdot 0.013\,\text{M}}{1.3 \times 10^{-5}\,\text{M} \cdot 0.025\,\text{M}}$$

$$\Delta G \approx 0$$

- Because enzyme keeps reaction at equilibrium!

Creatine Kinase in Muscle Cells

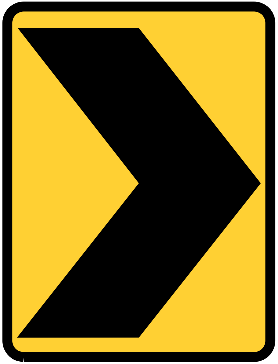
$$\text{ADP} + \text{CP} \rightleftharpoons \text{ATP} + \text{C} \qquad \Delta G^\circ = -13\,\text{kJ/mol}$$

- If 1 mM ATP is suddenly converted to ADP:
  4 mM ATP $\rightarrow$ 3 mM ATP
  0.013 mM ADP $\rightarrow$ 1 mM ADP
  25 mM creatine phosphate
  13 mM creatine
- Calculate ΔG for these concentrations:

$$\begin{aligned}
\Delta G &= \Delta G^\circ + RT \ln \frac{[\text{ATP}][\text{C}]}{[\text{ADP}][\text{CP}]} \\
&= -13\,\text{kJ/mol} + RT \ln \frac{0.003\,\text{M} \cdot 0.013\,\text{M}}{0.001\,\text{M} \cdot 0.025\,\text{M}} \\
&\approx -12\,\text{kJ/mol}
\end{aligned}$$

- Creatine phosphate provides emergency reserve of free energy.

Warning!



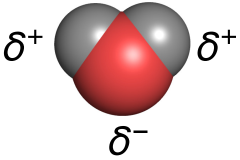
Direction Change

Water: What Makes it Special?

- Unusually high boiling temperature.
  - Boiling temperature is point where vapor pressure reaches atmospheric pressure.
  - Generally, boiling temperature reflects strength of interactions between molecules.
  - Generally, boiling temperature increases with size of molecules, because larger molecules have larger surfaces for interaction.
  - Boiling temperature of water is very high for its size.
- Melting temperature of solid (ice) is also relatively high for size.
- Does not mix well with many other liquids, especially hydrocarbons.

Water Molecules are Polar

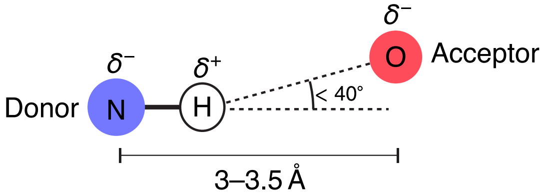
- Chemical bonds represent “sharing” of electrons between atoms.
- In some bonds, sharing is quite even: C-C, O-O, C-H
- Some elements are “greedy” for electrons: Electronegative elements: Oxygen and Nitrogen
- Some elements are “generous” with electrons: Electropositive elements: H, metals
- Water is particularly polar:



Oxygen has partial negative charge, and hydrogens have partial positive charges.

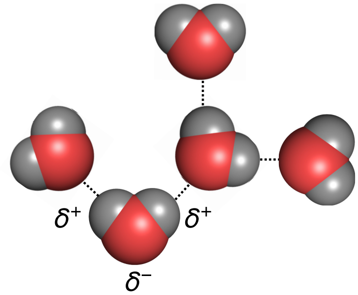
Hydrogen Bonds

- Form between a hydrogen atom covalently bonded to an electronegative atom and a second electronegative atom.



- Electronegative atoms are usually nitrogen or oxygen in biological molecules.
- Largely accounted for by electrostatic interaction between partial positive and negative charges, but there is probably also a small degree of covalent character to hydrogen bonds.
- Significant variability in geometry and strength of hydrogen bonds.

Hydrogen Bonds Between Water Molecules



- Each water molecule can act as a donor for two hydrogen bonds and an acceptor for two hydrogen bonds.
- In ice, each water molecule is hydrogen bonded to four others. Same geometry as carbon atoms in a diamond.
- What about in liquid water?

Clicker Question #3

How many hydrogen bonds does a water molecule form, on average, in liquid water at room temperature?

A) 0

B) 1

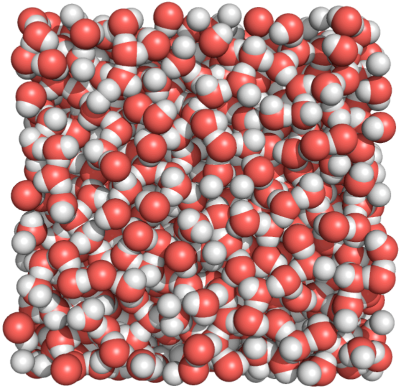
C) 2

D) 3

E) 4

All answers count for now.

Hydrogen Bonds in Liquid Water



Picture from simulation by
Prof. Valeria Molinero
U of U Chemistry Department.

- On average, each water molecule forms 3 hydrogen bonds at any instant.
- Explains high boiling point of water.
- Hydrogen bonds break and form constantly, giving water liquid properties.

A Simulation of 1,000 Water Molecules

(Water Movie)

- 4.5×10^{-12} s (4.5 ps) total time.
- 0.5 ps/frame

Simulation courtesy of Prof. Valeria Molinero, University of Utah

Ionization of Water

- A second consequence of polarity of water:

  Covalent O-H bonds break relatively easily

$$H_2O \rightleftharpoons H^+ + OH^-$$

- This is very unfavorable in vacuum or non-polar liquids.
- Interactions between H^+ or OH^- with polar water molecules stabilize the ions.
- H^+ or OH^- are in complexes with clusters of water molecules.
- Equilibrium constant for ionization:

$$K_{eq} = \frac{[H^+]_{eq}[OH^-]_{eq}}{[H_2O]_{eq}} = 1.8 \times 10^{-16}\,M$$

- By most standards, not a very favorable reaction at all, but H^+ and OH^- are potent!