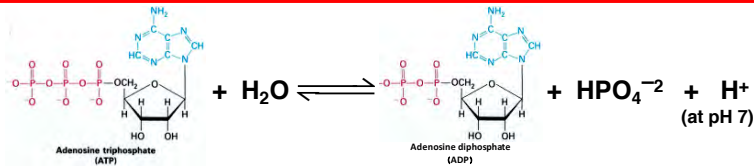


# Metabolism



**At equilibrium,  $\Delta G=0$**

$$\Delta G' = \Delta G^{\circ'} + RT \ln \frac{[C]^c [D]^d}{[A]^a [B]^b}$$

**BUT, if we are at equilibrium, this equation becomes:**

$$\Delta G' = \Delta G^{\circ'} + RT \ln K'_{eq}$$

$$\Delta G^{\circ'} = -RT \ln K'_{eq}$$

**For the above reaction (hydrolysis of ATP):**

$$\Delta G^{\circ'} = -7.3 \text{ kcal/mole}$$

**TABLE 13-3** Relationships among  $K'_{eq}$ ,  $\Delta G^{\circ'}$ , and the Direction of Chemical Reactions

When $K'_{eq}$ is ...	$\Delta G^{\circ'}$ is ...	Starting with all components at 1 M, the reaction ...
>1.0	negative	proceeds forward
1.0	zero	is at equilibrium
<1.0	positive	proceeds in reverse

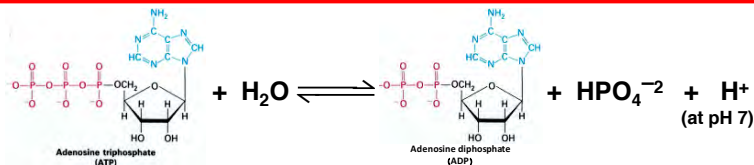
But, what is the actual  $\Delta G'$  of ATP Hydrolysis IN THE CELL?

**TABLE 13-2** Relationship between Equilibrium Constants and Standard Free-Energy Changes of Chemical Reactions

$K'_{eq}$	$\Delta G^{\circ'}$	
	(kJ/mol)	(kcal/mol)*
10 <sup>3</sup>	-17.1	-4.1
10 <sup>2</sup>	-11.4	-2.7
10 <sup>1</sup>	-5.7	-1.4
1	0.0	0.0
10 <sup>-1</sup>	5.7	1.4
10 <sup>-2</sup>	11.4	2.7
10 <sup>-3</sup>	17.1	4.1
10 <sup>-4</sup>	22.8	5.5
10 <sup>-5</sup>	28.5	6.9
10 <sup>-6</sup>	34.2	8.2

\* Although joules and kilojoules are the standard units of energy and are used throughout the text, biochemists and molecular biologists sometimes express  $\Delta G^{\circ'}$  values in kilocalories per mole. We have therefore included values in both kilojoules and kilocalories in this table and in Tables 13-4 and 13-6. To convert kilojoules to kilocalories, divide the number of kilojoules by 4.184.

# Metabolism



Go back the the equation:  $\Delta G' = \Delta G^{\circ'} + RT \ln \frac{[C]^c [D]^d}{[A]^a [B]^b}$

$$\Delta G = \Delta G^{\circ'} + RT \ln \frac{[MgADP^+][P_i]}{[MgATP^{2-}]}$$

At 25 °C:

$$\Delta G' = \Delta G^{\circ'} + RT \ln \frac{[ADP^3-][HPO_4^{2-}]}{[ATP^4-]}$$

$$\Delta G' = \Delta G^{\circ'} + 0.59 \ln \frac{[ADP^3-][HPO_4^{2-}]}{[ATP^4]}$$

$$\Delta G' = \Delta G^{\circ'} + 1.36 \log (1.5 \times 10^{-4})$$

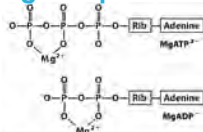
$$\Delta G' = (-7.3) + (-5.2)$$

$$\Delta G' = -12.5 \text{ kcal/mole}$$

WOW!

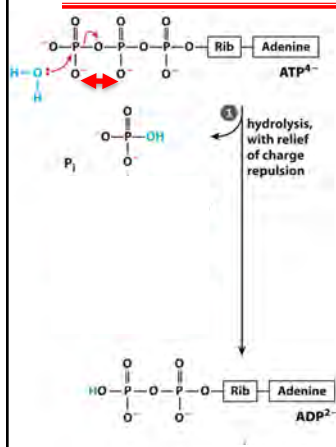
What makes the  $\Delta G'$  of ATP so high?

**$\Delta G^{\circ'}$  of ATP Hydrolysis is Mg<sup>++</sup> Dependent**



In RBCs =  $1.5 \times 10^{-4} \text{ M}$

# Metabolism



- Contributions of the incredible favorability of the ATP hydrolysis reaction:
  - Better charge separation in products (relief of charge repulsion)
  - More favorable resonance stabilization of products
  - Ionization of products (ADP or  $P_i$ )

Are there other compounds in the cell with high negative  $\Delta G^\circ$  of hydrolysis?

# Metabolism

**Acetyl phosphate**

$\Delta G^\circ = -11.8 \text{ kcal/mole}$

**Phosphocreatine**

$\Delta G^\circ = -10.3 \text{ kcal/mole}$

**Phosphoenolpyruvate (PEP)**

$\Delta G^\circ = -14.8 \text{ kcal/mole}$   
 $= -61.9 \text{ kJ/mole}$

**Adenosine triphosphate (ATP)**

Reaction type	$\Delta G^\circ$	
	(kJ/mol)	(kcal/mol)
<b>Hydrolysis reactions</b>		
Acetic anhydride + $H_2O \rightarrow 2 \text{ acetate}$	-91.1	-21.8
ATP + $H_2O \rightarrow ADP + P_i$	-30.5	-7.3
ATP + $H_2O \rightarrow AMP + PP_i$	-45.6	-10.9
$PP_i + H_2O \rightarrow 2P_i$	-19.2	-4.6
UDP-glucose + $H_2O \rightarrow UMP + \text{glucose 1-phosphate}$	-43.0	-10.3
<b>Esters</b>		
Ethyl acetate + $H_2O \rightarrow \text{ethanol} + \text{acetate}$	-19.6	-4.7
Glucose 6-phosphate + $H_2O \rightarrow \text{glucose} + P_i$	-13.8	-3.3
<b>Amides and peptides</b>		
Glutamine + $H_2O \rightarrow \text{glutamate} + NH_4^+$	-14.2	-3.4
Glycylglycine + $H_2O \rightarrow 2 \text{ glycine}$	-9.2	-2.2
<b>Glycosides</b>		
Maltose + $H_2O \rightarrow 2 \text{ glucose}$	-15.5	-3.7
Lactose + $H_2O \rightarrow \text{glucose} + \text{galactose}$	-15.9	-3.8
<b>Rearrangements</b>		
Glucose 1-phosphate $\rightarrow$ glucose 6-phosphate	-7.3	-1.7
Fructose 6-phosphate $\rightarrow$ glucose 6-phosphate	-1.7	-0.4
<b>Elimination of water</b>		
Malate $\rightarrow$ fumarate + $H_2O$	3.1	0.8
<b>Oxidations with molecular oxygen</b>		
Glucose + $6O_2 \rightarrow 6CO_2 + 6H_2O$	-2,840	-686
Palmitate + $23O_2 \rightarrow 16CO_2 + 16H_2O$	-9,770	-2,338

**PEP** +  $H_2O \rightarrow \text{Pyruvate (enol form)} + P_i$

**Pyruvate (enol form)**  $\xrightarrow{\text{tautomerization}}$  **Pyruvate (keto form)**

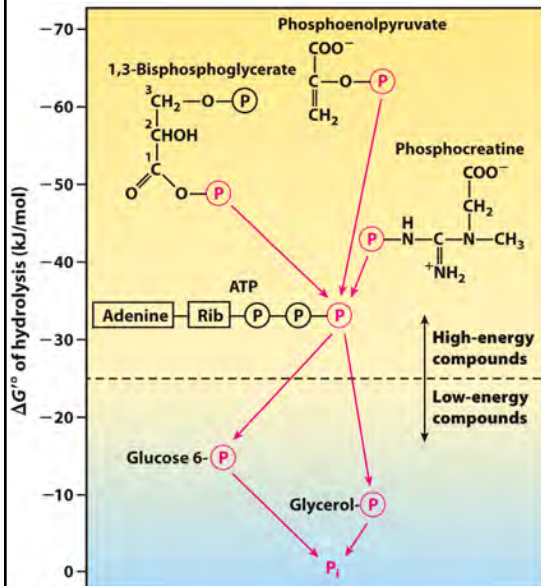
**PEP** +  $H_2O \rightarrow \text{pyruvate}^- + \text{HPO}_4^{2-}$   
 $\Delta G^\circ = -61.9 \text{ kJ/mol}$

Notice:

- anhydride or mixed anhydride bonds
- products have more resonance forms

2

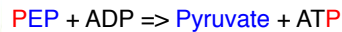
# Metabolism



## Phosphates: Ranking by the Standard Free Energy of Hydrolysis

Phosphate can be transferred from compounds with **higher**  $\Delta G^\circ$  to those with **lower**  $\Delta G^\circ$ .

Reactions such as:



are **favorable** and can be used to **synthesize ATP**.

# Metabolism

**TABLE 13-5** Total Concentrations of Adenine Nucleotides, Inorganic Phosphate, and Phosphocreatine in Some Cells

	Concentration (mM) <sup>a</sup>					
	ATP	ADP <sup>b</sup>	AMP	Energy Charge	$P_i$	PCr
Rat hepatocyte	3.38	1.32	0.29	0.81	4.8	0
Rat myocyte	8.05	0.93	0.04	0.94	8.05	28
Rat neuron	2.59	0.73	0.06	0.87	2.72	4.7
Human erythrocyte	2.25	0.25	0.02	0.94	1.65	0
<i>E. coli</i> cell	7.90	1.04	0.82	0.86	7.9	0

<sup>a</sup> For erythrocytes the concentrations are those of the cytosol (human erythrocytes lack a nucleus and mitochondria). In the other types of cells the data are for the entire cell contents, although the cytosol and the mitochondria have very different concentrations of ADP. PCr is phosphocreatine, discussed on p. 516.

<sup>b</sup> This value reflects total concentration; the true value for free ADP may be much lower (p. 509).

Cellular **ATP** concentration is usually far **above the equilibrium concentration**, making ATP a very potent source of chemical energy.

# Energy Coupling

## Energy Coupling

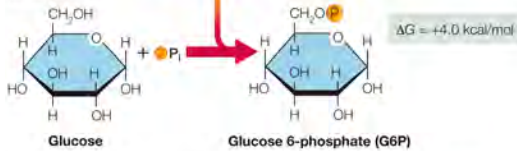
- Question is how do cells use these “high-energy compounds” to ensure that thermodynamically unfavorable reactions can become favorable?
- Chemical coupling of exergonic and endergonic reactions allows otherwise unfavorable reactions.
- TWO basic mechanisms:
  - e.g., The hydrolysis of the “high-energy” molecule (ATP), which is exergonic, is coupled with an otherwise endergonic reaction **during an enzyme mechanism**.
  - e.g., a series of otherwise endergonic reactions **during a metabolic pathway** are linked in the reaction series to an overwhelming exergonic reaction (all catalyzed by enzymes).

## Energy Coupling

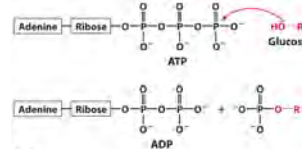
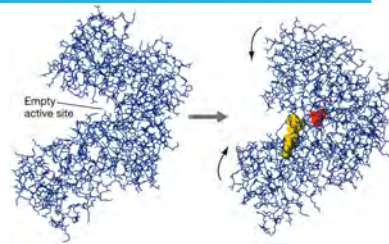
**Exergonic reaction**  
(releases energy)



**Endergonic reaction**  
(requires energy)



Net  $\Delta G = -3.3 \text{ kcal/mol}$



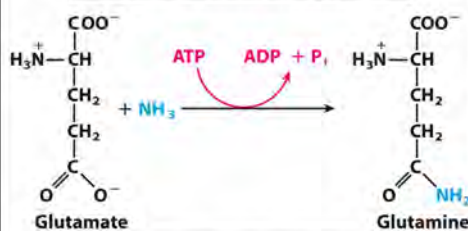
**Enzymatic reaction**

These two reactions take place at the same place (active site)  
Replace the H-O-H with H-O-R (glucose)

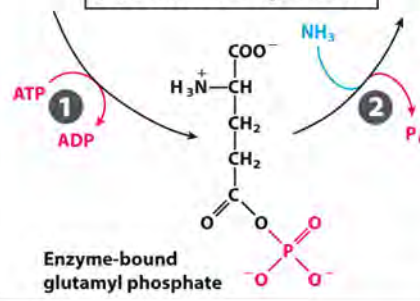


## Energy Coupling

(a) Written as a one-step reaction



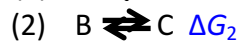
(b) Actual two-step reaction



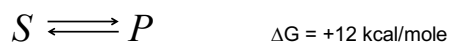
**Two-Step Group Transfers  
Facilitate ATP-Dependent  
Reactions**

## Energy Coupling

Standard free-energy changes are **additive**:



Coupling exergonic and endergonic reactions:



# Chemical Reactivity

## Chemical Reactivity

Most biochemical reactions fall within only a few categories:

- Cleavage and formation of **C–C bonds**
- Cleavage and formation of polar covalent bonds  
(**Addition-Elimination** Reactions)
  - nucleophilic substitution mechanism
  - addition–elimination mechanism
    - hydrolysis and condensation reactions
    - Eliminations (without cleavage)
- Internal **rearrangements**
- **Group transfers** ( $\text{H}^+$ ,  $\text{CH}_3$ ,  $\text{PO}_3^{2-}$ )
- **Oxidations-reductions** ( $e^-$  transfers)

## Chemical Reactivity

### Chemistry at Carbon: Homolytic versus Heterolytic Cleavage

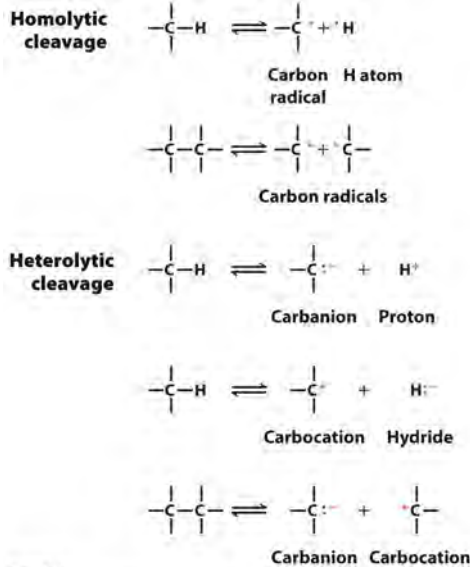
Homolytic  
cleavage

Heterolytic  
cleavage

- Covalent bonds can be broken in two ways.
- Homolytic cleavage is rare in biochemistry, but makes up some of the most interesting reactions.
- Heterolytic cleavage is common, but the products are highly unstable, dictating the chemistry that occurs: mostly carbanion chemistry

## Chemical Reactivity

### Chemistry at Carbon: Homolytic versus Heterolytic Cleavage





- Covalent bonds can be broken in two ways.
- Homolytic cleavage is rare in biochemistry, but makes up some of the most interesting reactions.
- Heterolytic cleavage is common, but the products are highly unstable, dictating the chemistry that occurs: mostly carbanion chemistry

## Chemical Reactivity

### Nucleophiles and Electrophiles in Biochemistry


#### Nucleophiles


  
Negatively charged oxygen (as in an unprotonated hydroxyl group or an ionized carboxylic acid)

  
Negatively charged sulfhydryl

  
Carbanion

  
Uncharged amine group

  
Imidazole

  
Hydroxide ion

**Definition:** *-phile* (suffix, Latin): to love, or demonstrate fondness towards


- Nucleophiles love nuclei. The nuclei of an atom contains positively charged particles (protons), so nucleophiles love to react with positive charges and are electron rich.
- Electrophiles love electrons. Electrons are negatively charged, so electrophiles must be positively charged.




# Chemical Reactivity

## Nucleophiles and Electrophiles in Biochemistry


### Nucleophiles


  
Negatively charged oxygen (as in an unprotonated hydroxyl group or an ionized carboxylic acid)

  
Negatively charged sulfhydryl


  
Carbanion


  
Uncharged amine group

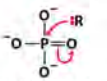
  
Imidazole

  
Hydroxide ion

### Electrophiles

  
Carbon atom of a carbonyl group (the more electronegative oxygen of the carbonyl group pulls electrons away from the carbon)

  
Protonated imine group (activated for nucleophilic attack at the carbon by protonation of the imine)

  
Phosphorus of a phosphate group

  
Proton

**Definition:** *-phile* (suffix, Latin): to love, or demonstrate fondness towards

- Nucleophiles love nuclei. The nuclei of an atom contains positively charged particles (protons), so nucleophiles love to react with positive charges and are electron rich.
- Electrophiles love electrons. Electrons are negatively charged, so electrophiles must be positively charged.

# Chemical Reactivity

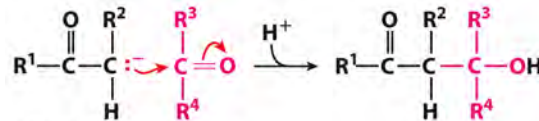
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    - hydrolysis and condensation reactions
    - Eliminations (without cleavage)
- Internal **rearrangements**
- **Group transfers** ( $\text{H}^+$ ,  $\text{CH}_3^+$ ,  $\text{PO}_3^{2-}$ )
- **Oxidations-reductions** ( $e^-$  transfers)

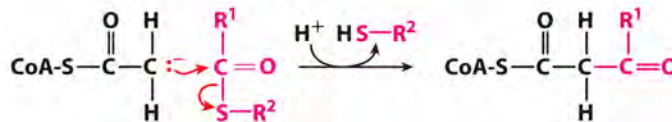
## Chemical Reactivity

C–C bonds

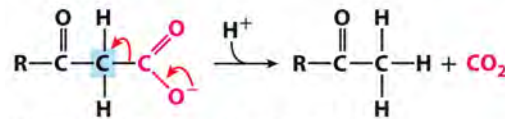
### Examples of Nucleophilic Carbon-Carbon Bond Formation Reactions



**Aldol condensation**



**Claisen ester condensation**



**Decarboxylation of a  $\beta$ -keto acid**

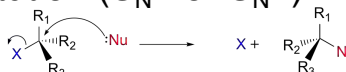
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## Chemical Reactivity Addition-Elimination

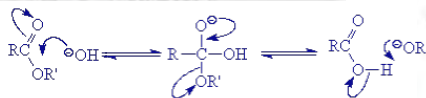
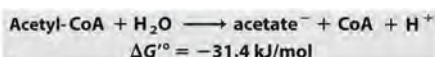
- Substitution from  $sp^3$  carbon proceeds normally via the nucleophilic substitution ( $S_N1$  or  $S_N2$ ) mechanism.



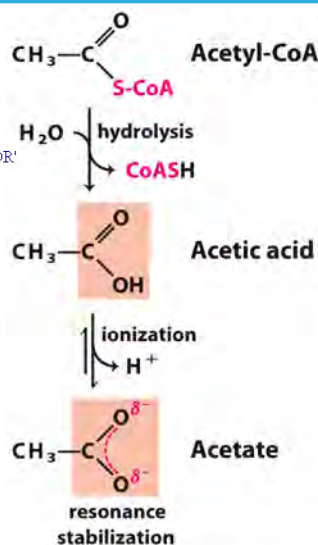
- Substitution from the  $sp^2$  carbon proceeds normally via the nucleophilic addition–elimination mechanism.
  - nucleophile adds to the  $sp^2$  center, giving a **tetrahedral intermediate**
  - leaving group eliminates from the tetrahedral intermediate
  - leaving group may pick up a proton

## Chemical Reactivity Addition-Elimination

### Hydrolysis of Thioesters

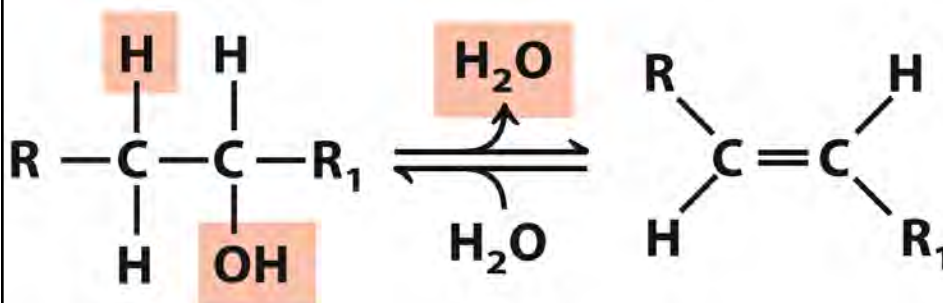


- Hydrolysis of thioesters is strongly favorable.
  - such as **acetyl-CoA**
- Acetyl-CoA is an important **donor of acyl groups**.
  - feeding two-carbon units into metabolic pathways
  - synthesis of fatty acids



## Chemical Reactivity Addition-Elimination

Elimination without  
Cleavage



Enzymes will do this stereo-specifically

## Chemical Reactivity

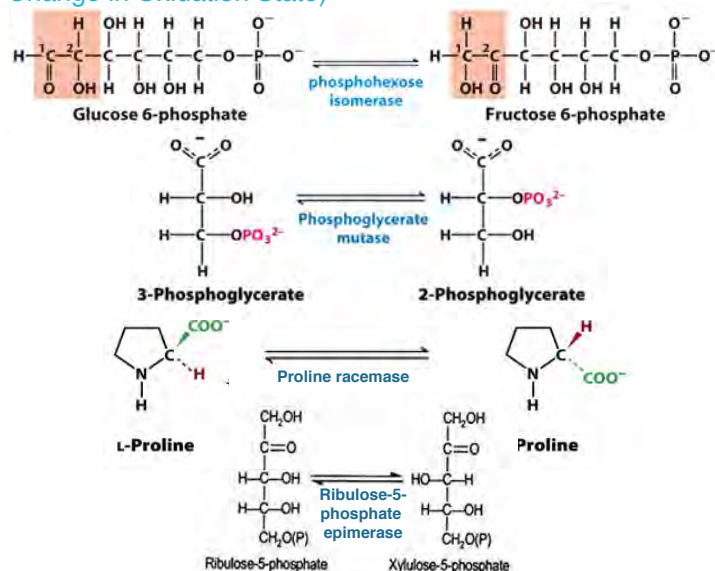
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    - hydrolysis and condensation reactions
    - Eliminations (without cleavage)
- Internal **rearrangements**
- Group transfers** (H<sup>+</sup>, CH<sub>3</sub><sup>+</sup>, PO<sub>3</sub><sup>2-</sup>)
- Oxidations-reductions** (e<sup>-</sup> transfers)

## Chemical Reactivity

Rearrangements

Isomerizations and Eliminations:  
(No Change in Oxidation State)



## Chemical Reactivity

Most biochemical reactions fall within only a few categories:

- Cleavage and formation of **C–C bonds**
- Cleavage and formation of polar covalent bonds (**Addition-Elimination Reactions**)
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- **Oxidations-reductions** ( $\text{e}^-$  transfers)

## Chemical Reactivity

### Group Transfers

- Proton transfer, very common
- Methyl transfer, various biosyntheses
- Acyl transfer, biosynthesis of fatty acids
- Glycosyl transfer, attachment of sugars
- Phosphoryl transfer, to activate metabolites
  - also important in signal transduction



$Z = R-OH$  (nucleophile)  
 $W = ADP$  (leaving group)



Substitution from  $sp^3$  phosphorous proceeds via the nucleophilic substitution (usually associative,  $S_N2$ -like) mechanism.

The nucleophile forms a partial bond to the phosphorous center, giving a **pentacovalent intermediate** or a penta-coordinated transition state.

## Chemical Reactivity

Most biochemical reactions fall within only a few categories:

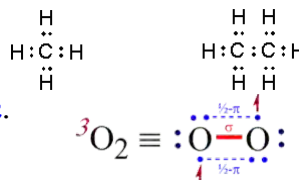
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- **Group transfers** ( $H^+$ ,  $CH_3^+$ ,  $PO_3^{2-}$ )
- **Oxidations-reductions** ( $e^-$  transfers)

## Chemical Reactivity

Re-dox

### Lessons from Quantum Chemistry

- Most organic molecules, including the reduced fuels, are in the **singlet spin state**.
  - All electrons are paired into electron pairs.
- Molecular oxygen is in the **triplet spin state**.
  - Two electrons are unpaired.
- Direct electron transfer from a singlet reduced species to a triplet-oxidizing species is quantum-mechanically **unfavorable**.
- This is why spontaneous oxidation of biomolecules does not occur readily, despite the exergonic reaction thermodynamics.
- Cofactors, such as transition metal ions and flavin adenine dinucleotide, are able to catalyze consecutive single-electron transfers needed for utilization of  $O_2$ .

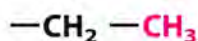


## Chemical Reactivity

Re-dox

### Oxidation-Reduction Reactions

Reduced organic compounds serve as **fuels** from which electrons can be stripped off during oxidation.



Alkane

Most energy

Relative  $\Delta G^{\circ}$  of oxidation per carbon (kcal/mol)

-196

-168

-125

-68

0

Least energy

## Chemical Reactivity

Re-dox

### Oxidation-Reduction Reactions

Reduced organic compounds serve as **fuels** from which electrons can be stripped off during oxidation.

$-\text{CH}_2 - \text{CH}_3$	Alkane	Most energy	Relative $\Delta G^{\circ}$ of oxidation per carbon (kcal/mol)
$-\text{CH}_2 - \text{CH}_2\text{OH}$	Alcohol		-196
$-\text{CH}_2 - \text{C}(=\text{O})\text{H(R)}$	Aldehyde (ketone)		-168
$-\text{CH}_2 - \text{C}(=\text{O})\text{OH}$	Carboxylic acid		-125
$\text{O}=\text{C}=\text{O}$	Carbon dioxide	Least energy	-68
			0

## Chemical Reactivity

Re-dox

### EXAMPLE: Reversible Oxidation of a Secondary Alcohol to a Ketone

- Many biochemical oxidation-reduction reactions involve transfer of **two electrons**.
- In order to keep charges in balance, proton transfer often accompanies electron transfer.
- In many **dehydrogenases**, the reaction proceeds by a stepwise transfers of **proton** ( $\text{H}^+$ ) and **hydride** ( $:\text{H}^-$ ).

