Some Key Chemistry Concepts
A brief summary of vitamins and cofactors
A brief interlude on digestion
Metabolic pathways
Ta daa…..glycolysis
Fig. 16-5a  Biologically important nucleophilic and electrophilic groups. (a) Nucleophiles.

(a) Nucleophiles

\[
\begin{align*}
\text{R} & \text{O} & \text{H} & \rightleftharpoons & \text{R} & \text{O} & : \\
\text{R} & \text{S} & \text{H} & \rightleftharpoons & \text{R} & \text{S} & : \\
\text{R} & \text{N} & \text{H}_3^+ & \rightleftharpoons & \text{R} & \text{N} & \text{H}_2 \\
\text{HN} & + & \text{NH} & \rightleftharpoons & \text{HN} & \text{N} & : \\
+H^+ & \text{Hydroxyl group} \\
+H^+ & \text{Sulfhydryl group} \\
+H^+ & \text{Amino group} \\
+H^+ & \text{Imidazole group}
\end{align*}
\]
Fig. 16-5b  Biologically important nucleophilic and electrophilic groups. (b) Electrophiles.

(b) Electrophiles

- $H^+$  Protons
- $M^{n+}$  Metal ions
- R\(\text{C=O}\)R'  Carbonyl carbon atom
- R\(\text{C=NH}^+\)R'  Cationic imine (Schiff base)
Four categories of biochemical reactions (according to Voet):

Group-transfer reactions
Redox reactions
Elimination / Isomerization / Rearrangement reactions
Making or Breaking - C–C – bonds
\[ R-C-X + Y^- \rightarrow [\begin{array}{c} O^- \\ Y \end{array}] \rightarrow R-C-Y + X^- \]
Fig. 16-7  The phosphoryltransfer reaction catalyzed by hexokinase.
Fig. 16-8 The molecular formula and **redox reactions** of the coenzyme flavin adenine dinucleotide (FAD).
Elimination, isomerization, rearrangement

Eliminations create double bonds; multiple mechanisms may be used.

Isomerization involves INTRAmolecular hydrogen shifts.

Rearrangements produced an altered CARBON skeleton.
Recall:

\[ \text{pKa for CH}_4 = 58 \]

\[ \text{pKa for CH}_3\text{COCH}_3 = 20 \]

Stabilization of carbanions in C-C chemistry

(a) \[\begin{array}{c}
\text{O}^\delta- \\
\text{C}^\delta+ \\
\end{array} \]

(b) \[\begin{array}{c}
\text{C} \\
\text{C} \\
\end{array} \]

(c) \[\begin{array}{c}
\text{O} \\
\text{R}_1 \\
\text{R}_2 \\
\text{R}_3 \\
\text{R}_4 \\
\end{array} \]

\[ \text{H}^+ \]

\[ \text{R}_1 \text{C} \text{C} \text{C} \text{C} \text{H} \text{O} \]

\[ \text{R}_1 \text{C} \text{C} \text{C} \text{C} \text{H} \text{O} \text{H} \]

Aldol condensation

\[ \begin{array}{c}
\text{O} \\
\text{R} \\
\text{C} \\
\text{C} \\
\text{H} \\
\text{H} \\
\end{array} \]

\[ \begin{array}{c}
\text{O} \\
\text{R} \\
\text{C} \\
\text{C} \\
\text{H} \\
\text{H} \\
\end{array} \]

\[ \text{H}^+ \]

\[ \text{R} \text{C} \text{C} \text{C} \text{H} + \text{CO}_2 \]

Decarboxylation of a \( \beta \)-keto acid
Schiff base (imine) formation

Nucleophilic attack on the carbonyl group by the amine leads to a dipolar intermediate.

Proton transfer occurs by removing a proton from N and adding a proton to O, giving a neutral carbinolamine.

An acid catalyst H—A protonates the oxygen to make the —OH a better leaving group.

Lone-pair electrons from nitrogen expel water, giving an iminium ion.

Deprotonation of the iminium ion regenerates the acid catalyst and yields the imine product.
Cofactors and coenzymes

Many proteins, including enzymes, function with only amino acid side chains; chymotrypsin is an example.

Some proteins require non-protein groups for proper structure or function. These groups are referred to as “prosthetic groups”; they may include glycosylations, lipids, metals, and more.

Enzymes may also require a non-protein group for activity; these are often called cofactors. Some enzyme cofactors may be metals, like Zn^{++} or Cu^{++}, but others may be larger, organic compounds. These groups can participate in chemistry that is not available to the side chains of the amino acids. Many times these cofactors are derived from vitamins. (Most vitamins must be ingested, we have lost the ability to synthesize them).

Some organic cofactors stay bound to an enzyme, and although they may be altered during reaction, they must be “rejuvenated” in situ. Flavins are like this.

Some vitamin derived cofactors turn over, like a substrate, with each reaction, and diffuse away. NAD/NADH is like this. These are sometimes called cosubstrates or coenzymes.
### Some Vitamins and Cofactors

Know this for the test

#### TABLE 8.9 Water-Soluble Vitamins

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Coenzyme</th>
<th>Typical reaction type</th>
<th>Consequences of deficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiamine (B₁)</td>
<td>Thiamine pyrophosphate</td>
<td>Aldehyde transfer Decarboxylation</td>
<td>Beriberi (weight loss, heart problems, neurological dysfunction)</td>
</tr>
<tr>
<td>Riboflavin (B₂)</td>
<td>Flavin adenine dinucleotide (FAD)</td>
<td>Oxidation–reduction</td>
<td>Cheliosis and angular stomatitis (lesions of the mouth), dermatitis</td>
</tr>
<tr>
<td>Pyridoxine (B₆)</td>
<td>Pyridoxal phosphate ≤</td>
<td>Group transfer to or from amino acids</td>
<td>Depression, confusion, convulsions</td>
</tr>
<tr>
<td>Nicotinic acid (niacin)</td>
<td>Nicotinamide adenine dinucleotide (NAD⁺)</td>
<td>Oxidation–reduction</td>
<td>Pellagra (dermatitis, depression, diarrhea)</td>
</tr>
<tr>
<td>Pantothenic acid</td>
<td>Coenzyme A</td>
<td>Acyl–group transfer</td>
<td>Hypertension</td>
</tr>
<tr>
<td>Biotin</td>
<td>Biotin–lysine complexes (biocytin)</td>
<td>ATP-dependent carboxylation and carboxyl-group transfer</td>
<td>Rash about the eyebrows, muscle pain, fatigue (rare)</td>
</tr>
<tr>
<td>Folic acid</td>
<td>Tetrahydrofolate</td>
<td>Transfer of one-carbon components; thymine synthesis</td>
<td>Anemia, neural-tube defects in development</td>
</tr>
<tr>
<td>B₁₂</td>
<td>5′-Deoxyadenosyl cobalamin</td>
<td>Transfer of methyl groups; intramolecular rearrangements</td>
<td>Anemia, pernicious anemia, methylmalonic acidosis</td>
</tr>
<tr>
<td>C (ascorbic acid)</td>
<td></td>
<td>Antioxidant</td>
<td>Scurvy (swollen and bleeding gums, subdermal hemorrhages)</td>
</tr>
</tbody>
</table>
A few vitamin structures

The vitamin is generally added to a “marker” group or a chemical “handle” to form a cofactor.
Some cofactor/coenzymes

Nicotinamide adenine dinucleotide, NAD⁺ (oxidation-reduction)
(NADP⁺)

Flavin adenine dinucleotide, FAD (oxidation-reduction)

Adenosine triphosphate, ATP (phosphorylation)

Coenzyme A (acyl transfer)
Some more cofactors

Thiamine diphosphate (decarboxylation)

Biotin (carboxylation)

S-Adenosylmethionine (methyl transfer)

Tetrahydrofolate (transfer of C₁ units)

Lipoic acid (acyl transfer)

Pyridoxal phosphate (amino acid metabolism)
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.
A redox example

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

\[
\text{NADH} + \text{H}^+ \rightarrow \text{NAD}^+
\]

![Chemical structures showing the redox reaction](image)
In complex organisms, metabolism is preceded by digestion and absorption!
Overview of Metabolism
Glycolysis

1. What is the role of this pathway?
   Convert 6-C sugars to 3-C pyruvate

2. What is the difference between respiration and fermentation?
   Aerobic vs. Anaerobic

3. Where is the pathway located, and how general is it?
   All enzymes are located in the cytosol, ~ universal

4. What are the characteristics of the metabolic intermediates?
   Phosphorylated intermediates

5. What are the “energy” gains or losses involved?
   ATP / NADH / FADH2 produced or consumed

6. What are the major regulatory steps in this pathway?

7. What are key mechanistic features utilized along the pathway?
Figure 17-3  Degradation of glucose via the glycolytic pathway.

Stage 1
- 6C sugars
- Stage 2
- 3C sugars
- 3C pyruvate
A stripped down Figure
Glycolysis (Greek = sweet-splitting)  
(Embden-Meyerhof; 1930's)

Production of **ATP** in absence of oxygen.  
**Breaks down glucose** (and other monosaccharides) into products which can yield ATP via oxidation.

Nearly **universal**; occurs in almost every cell of every organism.

**Probably 1st enzyme-catalyzed** catabolic system on earth -  
(During 1st 2 billion yrs, only prokaryotes existed in anaerobic atmosphere. Molecular O₂ was not introduced until after advent of bacterial photosynthesis.)

• 11 enzymes, all in cytosol
• all intermediates between glucose and pyruvate phosphorylated
Preparative Stage 1: Collection of simple hexoses, priming with phosphates for catabolism; and conversion of 6-C sugars into 3-C sugars.

1. glucose phosphorylation: hexokinase or glucokinase

\[
\alpha-D\text{-glucose} + \text{ATP} \quad \text{Mg}^{++} \quad \rightarrow \quad \text{ADP} + \alpha-D\text{-glucose-6-phosphate}
\]

- **hexokinase** = most widely distributed, most cells use it.
  - regulatory enzyme: feedback-inhibited by product
- **glucokinase** = predominate isozyme in liver.
  - requires higher glucose concentrations (~10 mM) for half-saturation and exhibits sigmoidal dependence on [glucose].
  - it is insensitive to physiological concentrations of product (glucose-6-P).
Fig. 17-5. Conformational changes in yeast hexokinase on binding glucose – engulfs glucose and excludes water.

Transfer of Pi to water would be energetically more favorable, and water could bind to C6 alcohol site, but the closed conformation makes specific transfer to glucose 40,000 x faster than hydrolysis.
Stage 1: collection of simple hexoses, priming with phosphates for catabolism; and conversion of 6-C sugars into 3-C sugars.

2. Glc-6-P to fructose-6-phosphate: phosphoglucose isomerase

\[ \alpha-D\text{-glucose-6-P} \quad \rightarrow \quad \alpha-D\text{-fructose-6-P} \]

- freely reversible in cell
- specific for ring substrates
- moving carbonyl to C2 sets up aldol cleavage rxn later
Fig. 17-6 Mechanism of phosphoglucone isomerase.

Notice which CH ionizes
Stage 1: collection of simple hexoses, priming with phosphates for catabolism; and conversion of 6-C sugars into 3-C sugars.

3. phosphorylation of fructose-6-P: phosphofructokinase

\[ \text{ATP} + \text{fructose-6-P Mg}^{++} \rightarrow \text{ADP} + \text{fructose-1,6-diphosphate} \]

- nucleophilic attack of C1-OH on electrophilic γ-phosphate of Mg++-ATP
- committed step - essentially irreversible (\(\Delta G^o = -14.2 \text{ kJ/mol}\))
- allosteric enzyme - key regulatory step in glycolysis
- activated by low E conditions / inhibited by high E conditions
Stage 1: collection of simple hexoses, priming with phosphates for catabolism; and conversion of 6-C sugars into 3-C sugars.

4. Cleavage of fructose-1,6-diphosphate to 2 triose phosphates: aldolase

reversible aldol cleavage / condensation reaction

\[
\text{fructose-1,6-diphosphate} \rightleftharpoons \text{dihydroxyacetone phosphate} + \text{glyceraldehyde-3-phosphate}
\]

Now we see why the isomerization was done; the aldol cleavage is \(\alpha-\beta\) to the carbonyl. If glucose was cleaved you’d produce C2 and C4 products.
Fig. 17-8 Mechanism of base-catalyzed aldol cleavage.

pKa of alcohol ~16

C-C bond breaking facilitated by carbanion stabilization.
Fig. 17-9 Mechanism of aldolase.

Enzyme attack creates an even better electron sink.
Stage 1: collection of simple hexoses, priming with phosphates for catabolism; and conversion of 6-C sugars into 3-C sugars.

5. Interconversion of triose phosphates: triosephosphate isomerase (classic β-barrel structure)

dihydroxyacetone phosphate $\rightleftharpoons$ glyceraldehyde-3-phosphate

- C-atoms 1 and 6 of glucose become C3 of glyceraldehyde-3-phosphate;
  
  $2 + 5 = \text{C2}; \quad 3 + 4 = \text{C1}$

- DHAP = 95% of equilibrium mixture - rxn pulled to right by removal of glyceraldehyde-3-phosphate in next rxn.
Transition state analog binds 100x better than substrates.

**Fig. 17-10**

Mechanism of TIM.

GAP•TIM Michaelis complex

DHAP•TIM Michaelis complex

Transition state

Enediol (or enediolate) intermediate
6. Oxid. of glyceraldehyde-3-P: glyceraldehyde phosphate dehydrogenase

\[
glyceraldehyde-3-P + NAD^+ + Pi \rightarrow 1,3\text{-diphosphoglycerate} + NADH + H^+\]

- **Conserves energy** of oxid. by coupling exergonic rxn to endergonic rxn; -

(aldehyde ox is \(~-43\text{KJ/mol}\); mixed anhydride formation unfavorable \(~49\text{ KJ/mol}\))

Energy is stored in acid anhydride bond; but overall \(\Delta G^\circ\) \(~+ 6\text{ kJ/mol}\)

- NADH produced carries electrons from glyceraldehyde-3-P to pyruvate (later in pathway)
Fig. 17-14 Mechanism of glyceraldehyde 3-P dehydrogenase.

Recall ester intermediate of serine proteases

Nucleophile is Pi, not water

Glyceraldehyde 3-phosphate

1,3-Bisphosphoglycerate (1,3-BPG)
Stage 2: $\times 2$

1 oxidation
2 substrate level phos.

7. Transfer of phosphate to ATP: phosphoglycerate kinase

$\text{1,3-diphosphoglycerate} + \text{ADP} \rightarrow \text{3-phosphoglycerate} + \text{ATP}$

- highly exergonic ($\Delta G^\circ = -18.8 \text{ kJ/mol}$) - pulls preceding rxn to completion:

- coupled rxn: \(\text{glyceraldehyde-3-P} + \text{Pi} + \text{ADP} + \text{NAD}^+ \rightarrow \text{3-phosphoglycerate} + \text{ATP} + \text{NADH} + \text{H}^+\)

\[
\Delta G^\circ = 6.7 - (-18.8) = -12.1 \text{kJ/mol}
\]

- Energy of oxid. of aldehyde to carboxylic acid conserved in form of ATP ($\times 2$).
Making of ATP - Substrate Level Phosphorylation

\[
\begin{align*}
\text{1,3-Bisphosphoglycerate} & \quad \text{Mg}^{2+} - \text{ADP} \\
\text{3-Phosphoglycerate} & \quad \text{Mg}^{2+} - \text{ATP}
\end{align*}
\]
Stage 2: x2
1 oxidation
2 substrate
level phos.

8. Conversion to 2-phosphoglycerate: phosphoglyceromutase

3-phosphoglycerate → 2-phosphoglycerate

- requires catalytic amounts of 2,3-BPG
Fig. 17-18 Mechanism of phosphoglycerate mutase.
Stage 2: x2
1 oxidation
2 substrate
level phos.

9. Dehydration to PEP: enolase

\[
\text{2-phosphoglycerate} \quad \rightarrow \quad \text{phosphoenolpyruvate} + \text{H}_2\text{O}
\]
- Produces high-energy phosphate bond: \(\Delta G^\circ\) for hydrolysis of 2PG = - 17.6 kJ/mol
- \(\Delta G^\circ\) for hydrolysis of PEP = - 61 kJ/mol = more than enough to make ATP in next step
The mechanism of Enolase

2-Phosphoglycerate (2 PG)
1 \rightarrow \text{fast}

Delocalized carbanion intermediate
2 \rightarrow \text{slow}

Phosphoenolpyruvate (PEP)
Stage 2: \[ x2 \]
1 oxidation
2 substrate
level phos.

10. Transfer of phosphate from PEP to ADP: pyruvate kinase

phosphoenolpyruvate + ADP Mg++ \[ \rightarrow \] pyruvate + ATP

- tautomerization of enol pyruvate is very exergonic
- since 2 mol PEP/glucose \( \uparrow\) 2 ATP/glucose at this step
- essentially irreversible under intracellular conditions
Making of ATP - Substrate Level Phosphorylation

Fig. 17-22  Mechanism of pyruvate kinase.

\[
\Delta G^\circ = +14.4 \text{ kJ/mol} \\
\Delta G^\circ = -46 \text{ kJ/mol}
\]

Overall \( \Delta G^\circ = -31.4 \text{ kJ/mol} \)
Fates of Pyruvate

Glucose
- glycolysis (10 successive reactions)

2 Pyruvate
- anaerobic conditions
- NADH
- 2 Ethanol + 2CO₂
  - Fermentation to alcohol in yeast
- aerobic conditions
- 2CO₂
- NADH
- 2 Acetyl-CoA
  - citric acid cycle
    - NADH
    - 4CO₂ + 4H₂O
      - Animal, plant, and many microbial cells under aerobic conditions

2 Lactate
- anaerobic conditions
- NADH
- 2 Lactate
  - Fermentation to lactate in vigorously contracting muscle, erythrocytes, some other cells, and in some microorganisms
END of this lecture