1.1 "Introduction to Biochemistry"

I. The science of biochemistry.

The ultimate goal of biochemistry is to explain all life processes in molecular detail. Because life processes are performed by organic molecules the discipline of biochemistry relies heavily on fundamental principles of organic chemistry and other basic sciences. It is of no surprise that the first "biochemists" actually were organic chemists who specialized in the chemistry of compounds derived from living organisms. The text provides an historical overview of some of the key contributions of the early chemists, and of modern 20th century biochemists who have lead the discipline to where it is today. Research endeavors such as the human genome project ultimately owe their success to basic discoveries about the structure of the DNA "double helix" by Watson & Crick and the development of DNA sequencing methods by Fredrick Sanger.

II. The chemical basis of life.

The biomolecules such as proteins that are present in living organisms are carbon-based compounds. Carbon is the third most abundant element in living organisms (relative abundance H > O > C > N > P > S). The most common ions are Ca^{+2}, K^+, Na^+, Mg^{+2}, and Cl^-. The properties of biomolecules, such as shape and chemical reactivity, are best described by the discipline of organic chemistry.

III. Biochemistry and evolution.

Biochemistry has greatly extended our knowledge of phylogeny and evolution that was acquired originally through the disciplines of comparative anatomy, population genetics and paleontology. In fact, only through biochemistry have we come to appreciate that all living organisms are similar at the molecular level. Namely, they share similar means of replication, cellular structure, and often energy utilization & production. For this reason, much of what we can learn about simple organisms such as *Escherichia coli* can be applied to the study of higher organisms such as us.

The similarity of organisms at a molecular level indicates that all are derived from a common ancestor. Carl Woese determined by comparisons of ribosomal RNA (rRNA) sequences that it is possible to construct a highly accurate tree of life showing the evolutionary relationship between all life forms. rRNA analysis has proven that living organisms are best divided into 3 domains of life--the Archaea, the Bacteria, and the Eukarya. At the end of the course we will discuss the properties of one of the most important enzymes used in modern biochemistry laboratories, Taq polymerase. This enzyme is derived from the Archaean species *Thermus aquaticus*, which was isolated from a hot spring located in Yellowstone Natl. Pk. One common use of this enzyme is in sequencing rRNA genes from newly isolated microorganisms.

1.1.i. Water as a biological solvent

I. Introduction.

While modern biochemistry tends to focus on the structure and function of molecules such as proteins and DNA, it is important to keep in mind that bio molecular structure and function are dictated by the properties of the medium in which they are dissolved. Therefore, this chapter presents an overview of the properties of water that are germane to the structure and function of biomolecules. As an illustration of the importance of water in biological systems, consider the formation of biological membranes. Cell membranes ultimately form due to the fact that the acyl chains of glycerophospholipids are not soluble in water. As a consequence, glycerophospholipids and other membrane lipids cluster together leading to structures such the cytoplasmic membrane and membranes of organelles. In this chapter, we will review fundamental properties of water such as solvation of polar and nonpolar molecules, water ionization and pH, and acid-base chemistry and buffering systems. These topics are essential for understanding everything that will be discussed in later chapters of the text.

II. General properties of water molecules.

The oxygen atom in a water molecule has an sp^3 arrangement of bonding orbitals in which the 2 H atoms and 2 unshared pairs of electrons are located in a tetrahedral arrangement around the oxygen. This arrangement results in a net dipole in which the end of the molecule containing the unshared electrons has partial negative character and the end containing the 2 hydrogens has partial positive character. In addition, each H-O- bond also has dipolar character due to unequal sharing of electrons between hydrogen and oxygen. Due to the fact that a net dipole exists in individual water molecules, water is regarded as a polar solvent.

III. H-bonding in water.

Neighboring molecules in bulk water are held together by non-covalent bonds known as H-bonds. In the figure, the H bond is the non-covalent attraction (dashed line) between the partially positively charged H atom attached to the left oxygen atom and one of the unshared electron pairs (not shown) of the oxygen atom on the right. Each water molecule has 2 unshared electron pairs and 2 hydrogens that can participate in H-bonding. Thus, each water molecule can H-bond to 4 neighbors. Since sp^3 molecular orbitals are tetrahedral oriented, neighboring water molecules surrounding a given water molecule are located in a tetrahedral arrangement. In ice, water molecules are organized in a rigid, precisely tetrahedral crystalline lattice where each molecule is H-bonded to 4 others. In liquid water, each water molecule is H-bonded to ~3.4 others on average. Local groups, i.e., "flickering clusters," of molecules only exist for nanoseconds. While a roughly tetrahedral arrangement of molecules is present, liquid water is denser than

ice because the somewhat irregular packing of molecules allows them to fit together a bit closer. Due to extensive H-bonding, water is highly cohesive. The cohesiveness of molecules confers a high melting point and boiling point in spite of the low molecular weight of water (18 g/mol). The high specific heat and heat of evaporation make water an excellent thermal buffer for actively metabolizing cells and tissues. It also explains why cold water can quickly conduct heat away from a swimmer leading to hypothermia and possibly death.

IV. Behavior of ionic and polar substances in water.

Because water molecules are polar, ionic compounds (electrolytes) and polar molecules are relatively soluble in water. Substances that can dissolve readily in water are referred to as hydrophilic. For salts (e.g., NaCl), both the cationic (Na^+) and anionic (Cl^-) components of the salt can be solvated via interactions with the negative and positive, respectively, ends of dipolar water molecules. Because the interactions are energetically favorable, the salt dissolves. Dissolved ions are considered to be "solvated" or "hydrated." The shells of surrounding water molecules shield the ions preventing them from strongly interacting and reforming the crystal. Water molecules also form H-bonds to polar functional groups in polar biomolecules such as sugars and amino acids. The different types of H-bonds that can form are discussed below. It should be noted that many biomolecules contain a combination of polar and nonpolar groups. Thus, the actual solubility of biomolecules is quite variable and depends on the relative proportions of polar and nonpolar regions.

V. Behavior of nonpolar substances.

Nonpolar substances are relatively insoluble in water and therefore are referred to as hydrophobic. Such molecules typically are hydrocarbons containing methylene, methyl, and aromatic ring functional groups. They generally lack polar groups that can interact with water molecules. Because water molecules cannot form H-bonds to a nonpolar substance, water molecules become highly ordered in the immediate vicinity of the compound forming ice-like bonds to one another. Indeed, cage-like structures known as clathrates are formed which can be viewed as rigid geodesic domes surrounding the nonpolar molecule. All this structuring decreases the disorder or entropy of the water, which is an energetically unfavorable process. To avoid this situation as much as possible, the suspended hydrophobic substances coalesce which reduces the surface area of the nonpolar molecules in contact with water. The term hydrophobic interactions refer to the clustering together of nonpolar molecules such as membrane lipids to avoid the entropically unfavorable process of ordering neighboring water molecules. It is important to note that hydrophobic interactions are not a type of chemical bond.

One other important class of molecules--the amphiphiles--deserves mention. These molecules have significant proportions of both hydrophilic and hydrophobic functional groups. Typical examples are detergents such as sodium dodecyl sulfate (SDS) which contains a highly water soluble sulfate group and a very insoluble 12-carbon alkyl group. This schizophrenic combination

results in the hydrocarbon chains clustering together away from water contact when SDS is added to water. In this case, the clusters formed are spherical structures known as micelles. At an air-water surface SDS molecules actually line up with their hydrocarbon tails pointing up into the air and the sulfate groups in contact with water. SDS is a useful detergent. Its hydrocarbon tail will bind to nonpolar surfaces, such as greasy dirt, and dissolve it within the interior of the micelle. After the dirt-filled micelles are suspended in water by agitation, the dirt and detergent can be rinsed away.

VI. Noncovalent interactions in biomolecules.

Weak, reversible bonds (noncovalent bonds or noncovalent interactions) mediate interactions between biomolecules. Noncovalent bonds are "individually weak, but collectively strong" and together stabilize the complex structures of biomolecules such as proteins. However, because they are individually weak, biomolecules exhibit flexibility which is important in processes such as enzyme catalysis. Furthermore, non-covalent interactions allow reversible binding of small biomolecules to enzymes and nucleic acids. Generally, noncovalent interactions are less than 1/10 th as strong as covalent bonds such as the -C-H bond. The general properties of each of type of noncovalent interaction and the energy required to break them (i.e., the strength) are summarized .

A. Charge-charge interactions.

Charge-charge interactions occur between oppositely charged functional groups or ions. These bonds are also known as ion pairing interactions and salt-bridges. The strength of these bonds is inversely dependent on the square of the distance separating the charges. Strength also depends on the medium in which they occur, with polar media such as water weakening interactions through solvation of interacting ions. Repulsive forces between like charges also can play an important role in biological processes.

B. H-bonds.

The H-bonds that occur between water molecules are just one example of the many types found in biomolecules. In general, an H-bond is defined as a dipolar attraction between the hydrogen atom attached to one electronegative atom, and a second electronegative atom. The H atom must be covalently bonded to an electronegative atom such as O or N to generate a molecular dipole. The atom with the covalently bound hydrogen atom is called the hydrogen donor, and the other atom is the hydrogen acceptor. The distance between the two electronegative atoms in an H-bond is ~0.3 nm (3 Å). H-bond strength is highly dependent on the alignment of molecular orbitals in the interacting molecules and is strongest when they are lined up properly. As a result, H-bonds are very important in establishing specificity in molecular interactions, e.g., A-T and G-C base pairing in DNA .

C. van der Waals forces.

These forces are attractions between oppositely oriented dipoles that are transiently induced in the electron clouds of closely interacting molecules. The strength of these forces is maximal when the interacting molecules are just touching. In fact, these forces become destabilizing and push molecules apart if molecules are compressed more tightly together. Note that the van der Waals contact radius is defined as the distance at which the attraction of molecules is maximal. van der Waals forces typically are the weakest of the noncovalent interactions. However, van der Waals bonds often are important in the packing of amino acids inside a folded protein and in the interactions between adjacent bases stacked within the DNA double helix. They also can mediate specific interactions because they become collectively strong if the interacting molecules have precisely complementary shapes and can approach one another closely.

VII. Water is a nucleophile.

Water often is a reactant in biochemical reactions. The unshared pairs of electrons in water molecules can behave as nucleophiles which can attack an electrophilic center in another molecule. A good example where water serves as a nucleophile is in the hydrolysis of peptide bonds. Although this is a favorable reaction, peptide bonds are actually quite stable due to the fact that the activation energy for this reaction is quite high. Thus the reaction is very slow at physiological temperatures and pH unless catalyzed by an enzyme.

VIII. Ionization of water.

As a prelude to our discussion of pH, we need to discuss the ionization of water, as it is through this reaction that solution pH ultimately is established. A water molecule has a slight tendency to undergo a dissociation reaction whereby a proton is lost to another water molecule. The products of this reaction are a hydronium ion (H_3O^+) and a hydroxyl ion (OH^-).

$$H_2O + H_2O \rightleftharpoons H_3O^+ + OH^-$$

A hydronium ion can donate its proton to another molecule and hence is considered to be an acid (proton donor). A hydroxyl ion can accept a proton from an acid and thus is called a base (proton acceptor). The ionization reaction commonly is written as

$$H_2O \rightleftharpoons H^+ + OH^-$$
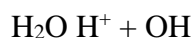
Water has a finite and defined capacity to ionize, and the ionization process has a characteristic equilibrium constant at a given temperature.

$[H^+][OH^-]$ $K_{eq} =$ $[H_2O]$

K_{eq} for water has been experimentally determined by measuring the electrical conductivity of pure water. (Note, electrical conductivity is proportional to the levels of ions in the water). The value for $K_{eq} = 1.8 \times 10^{-16}$ M. This K_{eq} value and the value of the concentration of water ($[H_2O]$ = 55.5 M) can be substituted into the equilibrium equation to derive another equation which specifies the amounts of $[H^+]$ and $[OH^-]$ in any water sample or biochemical buffer:

$[H^+][OH^-]$ 1.8×10^{-16} M =   (55 M)

$K_W = (55.5$ M$)(1.8 \times 10^{-16}$ M$) = [H^+][OH^-]$

$K_W = 1 \times 10^{-14}$ M$^2 = [H^+][OH^-]$

The constant, K_W, is called the ion product of water. The derivation indicates that the product of the $[H^+]$ and $[OH^-]$ concentrations in any water sample or buffer always will equal 1×10^{-14} M^2.

This result leads directly to a definition of a "neutral solution" and to a definition of the pH scale. A neutral solution is defined as one in which $[H^+] = [OH^-]$. When these concentrations   are equivalent, $[H^+] = [OH^-] = 1 \times 10^{-7}$ M. Due to the fact that this equilibrium reaction always is obeyed, the addition of a base which consumes protons leads to an excess of hydroxyl ions and a basic solution. Likewise the addition of an acid which consumes hydroxyl ions leads to an excess   of hydronium ions and an acidic solution.