Chapter 2: The Nature of Noncovalent Interactions

OVERVIEW OF SEMESTER

In this semester, we will be studying structural biochemistry. Structural biochemistry describes the different biomolecules that make up cells, and describe how the structures contribute to function. Biomolecules tend to be large molecules, referred to as macromolecules, and hence the structures of these molecules are likely to be different from the structures of molecules you have encountered in the past. In some sense, structural biochemistry is in essence the application of Physical Chemistry to problems of biological interest. Next semester, if you continue on with CHM 4622, you will be studying metabolism. Metabolic biochemistry is the application of Organic Chemistry to problems of biological interest.

In the area of Structural Biochemistry, there are two predominant general concepts that will recur in our discussions. The first is the influence of water upon the structures of macromolecules. Life is a big solubility problem, and the trials and tribulations of solubilizing macromolecules is one of the major topics in the understanding of how our cells work. The second area is molecular recognition. Our cells are one big puzzle, so to speak, and how one molecules fits together with a second molecule is of primary importance in understanding the assembly of cells and also reproduction. Let’s begin our semester with a brief introduction into these two topics.

Noncovalent Interactions

Before we talk about water, we need to review a little physical chemistry describing noncovalent reactions between molecules. Biochemistry is based upon these weak, noncovalent attractive forces between molecules. The simplest form of these interactions are the interactions between two charged molecules. Molecules of similar charge repel each other, while molecules of opposite charge attract. This is described mathematically by Coulomb’s law, which says that the force between a pair of charges separated in a vacuum by a distance r is described by:

\[ F = \frac{k|q_1q_2|}{r^2} \]
This describes the force in a vacuum; in a solution, the force is modified by the
dielectric constant of the medium:
\[ \mathbf{F} = k(q_1q_2)/\varepsilon r^2 \]
The higher the dielectric constant of the medium, the weaker the forces between
charges. Since water has a relatively high dielectric constant (about 80), the force here
is weaker than in an organic solvent with a dielectric constant of 1-5.

Permanent and induced dipoles can also interact with each other. Imagine a bar
magnet with a positive and negative pole. Two bar magnets will align so that the
negative pole of one will interact with the positive pole of the other, and vice versa.
The strength of interactions involving dipoles is very distance dependent. For example,
the energy of a dipole/dipole interaction is proportional to the cube of the distance
between the two dipoles. The magnitude of the force between different types of
charges and dipoles are described in Figure 2.2 in your text (Show powerpoint).

Water in Biological Systems

Why do we need to discuss water? Our cells, besides being made up of the
components I’ve just described, also contain a lot of water. Our cells contain between
70-90% water. As we will find out as the semester progresses, water is the major
influence on the structures adapted by biological structures such as membranes, and
macromolecules such as proteins or DNA. Therefore, to fully understand these
structures and what holds them together, we must first review the physical and
chemical properties of water, and how these relate to the normal function of biological
systems.

Let’s talk for a minute about water. Water has the molecular formula H\textsubscript{2}O (Alas
for little willie...). Let’s see how much organic chemistry you remember. What is the
three dimensional structure of water? (BENT) Why is the water molecule bent (sp\textsubscript{3}
hybridization of the oxygen electrons). How about the dipole moment of water? Does
water have a dipole moment? (YES) In what direction on the water molecule does the
dipole moment lie? The magnitude of the dipole moment of water is $1.87 \times 10^{-18}$ esu.
This is not very large, but it does mean that the molecule has a permanent charge.
distribution within it. The protons have a partial positive charge and the electrons a partial negative charge.

This dipole moment means that water can interact with other polar or ionic substances to form hydrogen bonds. A hydrogen bond is not quite a covalent bond but more than a simple electrostatic interaction. In a hydrogen bond, one of the hydrogens of water is "shared" with a hydrogen bond acceptor on another molecule, or conversely, a proton on some other molecule is "shared" with the oxygen on the water. These hydrogen bonds are what make water such a cohesive liquid. The hydrogen bonds give water the large surface tension that is has - if you look at a graduated cylinder of water, or some other narrow container of water, you see that the level of water across the narrow container is not level, that it dips in the middle and runs up the sides a little bit.

If you take molecules that do not have polar characteristics, these do not solubilize well in water. Suppose that I add a a blob of vegetable shortening to a beaker of water. What do you expect will happen to the shortening? Nothing - as you might guess, vegetable shortening is not soluble in water. It is predominantly nonpolar and hydrophobic. Suppose I repeat the exercise with urea; what would you expect? Urea is very soluble in water. Urea is a polar and hydrophilic molecule. Finally, suppose that you attempt to dissolve sodium dodecyl sulfate in water? Sodium dodecyl sulfate is moderately soluble in water, but not in the traditional sense. SDS is amphipathic - it has a polar part and a nonpolar part. Part of the molecule wants to dissolve in water, and part doesn't want to. As a result, an amphiphilic molecule like SDS will form a structure called a micelle in water. A micelle is usually a ball of the amphipathic molecule where the part of the molecules which doesn't like water are huddled in the middle of the ball, while the part of the molecule that does like water is found on the surface of the ball. In this way, both parts of the molecule are kept happy in aqueous solutions.

Effect of water on biological structure

Water profoundly affects the structure of macromolecules (large molecules) which are dissolved or suspended in water. Let me give you two examples.
Membranes are comprised of molecules called lipids, which have a polar end and a nonpolar end. In an organic solvent such as chloroform, the lipids float free in solution. The solvent is polar enough to keep the polar part of the molecule solvated, but nonpolar enough to keep the nonpolar part happy. However, if you put the same lipids into a polar aqueous solvent, a problem arises. The water still likes the polar part of the lipid, but the nonpolar part does not dissolve in the water. The lipid overcomes its unhappiness by forming a membrane. In a membrane, the nonpolar parts aggregate together, avoiding interaction with water, but the polar parts face the water solution and are also happy. If you want to dissolve a membrane, and hence dissolve a cell, add either detergent or organic solvent. The detergent or solvent changes the way that water interacts with the lipids, disrupting their structure.

A second example is proteins. Proteins are nicely soluble in water because water will interact with polar and charged parts of the protein, solvating these and in effect keeping them from interacting with other proteins and cell components. [SHOW OVERHEAD]. But what happens when you remove the water? If you remove the water slowly, under controlled conditions, then the water gets eliminated from the protein surface; protein-protein contacts become allowed, resulting in crystals of protein. Biochemists take advantage of protein crystals by protein crystallography, which is what I did on sabbatical leave a few years ago.

Proteins and membranes assume the structures that they do because of the **hydrophobic effect**. In simple terms, the hydrophobic effect says that like molecules, or like parts of molecules, will associate with one another, while unlike parts of molecules will not. On this overhead is a picture of the protein myoglobin. Myoglobin is a protein in muscle involved in oxygen storage. It is prevalent in the muscles of diving mammals like dolphins and whales, and allows them to stay under water for longer periods of time. What I want to point out from this picture is the topology of charges in this molecule. As we’ll see in a few minutes, proteins contain nonpolar and polar parts. Myoglobin is shaped in such a way that the polar parts are on the surface of this ball-shaped protein. The nonpolar parts are in the middle. The nonpolar parts dislike water, and aggregate in the center of the ball to avoid the water. The polar
parts like the water, and will sit on the surface and interact with the water molecule while shielding the nonpolar parts from water. If you add an alcohol or some other nonpolar solvent to the protein solution, the nonpolar parts in the middle will want to get out, while the polar parts will wish to hide. The protein will change its shape and as a result will no longer function correctly.

**Molecular Recognition**

Suppose that you go to a party. You look around and recognize some friends of yours. You move across the room and hang with them. As a result of hanging with them, you have someone to talk to, someone with common interests, people who might introduce you to new friends, etc. That is, if you divide the process up into three parts, you have recognition, followed by association, followed by response.

Life works in about the same way. Suppose you are a liver cell looking for guidance on what the blood sugar level is like. A hormone floats by, either glucagon or insulin, that you recognize. This hormone sticks to the surface of the liver cell (association), and the association leads to a response by the liver cell. If glucagon is bound, the cell releases sugar; if insulin is bound, the cell accumulates sugar from the blood.

The first step in most biological processes is recognition. The molecules in our cells, as a rule, form specific associations with a very small number of other molecules. To continue the glucose analogy, our cells have the ability to convert many different sugars into energy. Several different six carbon sugars that we use for energy are glucose, fructose, mannose, and galactose. Each of these sugars is initially recognized by a different metabolic enzyme, even though the structural differences between the sugars are very slight.

Many diseases begin by improper recognition or deception by the disease causing agent. HIV is a viral disease that infects T-cells in our immune system. The infectious process begins by the binding of the HIV virus particle to proteins on the surface of the T-cell normally designed to recognize small effector molecules called cytokines. The surface of the virus has a structure that fools the T-cell that it is a cytokine. Upon binding to the T-cell, the T-cell too late finds out that a mistake has
been made; the cell is infected and that’s that.
In the first part of the semester, we will first look at the structures of small molecules that are assembled into larger molecules. We will take some time to see how the assembled smaller molecules make a surface that leads to the specific recognition of other molecules.

**Acid-Base Properties**

The property of water which we will be most interested in to begin is its **ionization properties**. Because the shared electrons of the oxygen/hydrogen bond are so tightly pulled towards the oxygen, there is a tendency for the water molecule to lose a proton to a neighboring water molecule to which the proton is hydrogen bonded:

\[
2 \text{H}_2\text{O} \rightleftharpoons \text{OH}^- + \text{H}_3\text{O}^+
\]

The products of the reaction are hydroxide ions and hydronium ions. If you have a liter of pure water at 25°C, at any given time there are only \(1 \times 10^{-7}\) moles of hydroxide ions and hydronium ions in solution. Like any chemical reaction, the ionization of water is an equilibrium process, and thus can be expressed like this. Any equilibrium process can be described by an **equilibrium constant**, which is mathematically the product of the products divided by the product of the reactants:

\[
K_{eq} = [\text{H}^+] [\text{OH}^-] / [\text{H}_2\text{O}]
\]

Here I’ve used the proton as an abbreviation for the hydronium ion. The hydronium ion is somewhat a make believe entity anyway, since both it and the hydroxide ion are extensively hydrated. The average stoichiometry of the final ion is uncertain, and exact stoichiometry at any given moment in time certainly changes rapidly.

Anyway, getting back to this equation, we know that the concentration of
pure water is 55.5 M. Since the concentration of the products are both 10^{-7}, we can assume that the ionization does not significantly change the concentration of water, so that the equation may be expressed as

\[(55.5)K_{eq} = [H^+] [OH^-]\]

The quantity \((55.5)K_{eq}\), the sum of two constants, is replaced by another constant:

\[(55.5)K_{eq} = K_w\]

where \(K_w\) is called the ion product of water. The value of \(K_w\) at 25°C is 1 x 10^{-14}.

\(K_w\) is the basis for the pH scale of water ionization. The pH scale, which I'm sure you're all familiar with, provides a convenient method of expressing the concentration of hydrogen ions (and therefore hydroxide ions) in aqueous solutions. pH is defined as

\[pH = \log_{10} \frac{1}{[H^+]} = -\log_{10} [H^+]\]

A corresponding quantity can be defined to describe the concentration of hydroxide ions in solution:

\[pOH = \log_{10} \frac{1}{[OH^-]} = -\log_{10} [OH^-]\]

In neutral solution, the concentration of hydrogen ions and hydroxide ions are both equal to 10^{-7} M; therefore the pH of a neutral solution is \(- \log [10^{-7}] = 7.0\).

ACIDS AND BASES

Let's take a minute to review a bit about acids and bases. What is an acid? The most commonly used definition of acids and bases in aqueous systems was developed by Bronsted and Lowry. By their definition, an acid is a proton donor and a base is a proton acceptor. An acid/base reaction always involves a conjugate acid/base
pair, which is made up of the proton donor and the proton acceptor. For example, acetic acid is an acid: it is a proton donor, while acetate ion is a base: it is a proton acceptor.

\[
\begin{align*}
\text{H}_3\text{C} & \text{C} & \text{O} & \text{H} \\
\text{H}_3\text{C} & \text{C} & \text{O} & \text{O} & \text{H}^+ \\
\end{align*}
\]

In water, the hydroxide ion is the proton acceptor or base, the hydronium ion is the proton donor.

If an acid is put into water, the water can act as the proton acceptor, forming hydronium ions. The extent to which an acid donates its proton(s) to water depends upon the relative attraction of the conjugate base of the acid to protons as compared to water. Acids which only have a slight tendency to donate their protons to water are termed \textit{weak acids}; those which readily donate their protons to water are termed \textit{strong acids}. The tendency for an acid to donate its proton to water is described by its \textit{dissociation constant}:

\[
\text{HA} + \text{H}_2\text{O} \rightleftharpoons \text{H}_3\text{O}^+ + \text{A}^- \\
K = [\text{H}^+] [\text{A}^-] / [\text{HA}] [\text{H}_2\text{O}]
\]

By convention, the concentration of water is eliminated. Since the process we are describing is the dissociation of the acid, the constant describing this process is called the \textit{acid dissociation constant} and is notated \(K_a\). This is sometimes indicated as \(K_a'\), to indicate that the dissociation constant used by the biochemist is not corrected for nonideality and ionic strength, as the physical chemist would routinely do.

This property is one of the joys of biochemistry. The physical chemist is worried about every little calculation and detail that might cloud his/her results a little. The biochemist has to work with live preparations. Each preparation is at least a little
different from each other. These differences always introduce much larger errors than
would be introduced by nonideal behavior. And these calculations are a little more
involved. So we just ignore them and go blissfully on with our studies.

For weak acids, the $K_a$'s are often very small numbers. Again, to express the
$K_a$ in a more managable fashion, the -logarithm of the equilibrium constant is taken and
reported as the $pK_a$, analogous to the pH.

**ACID-BASE TITRATION CURVES**

In many situations in chemistry and biochemistry, the need arises to mix acids
and bases together. The obvious questions are:

1. Can we use the controlled mixing of an unknown acid and a known base (or vice
   versa) to provide information about the unknown compound?
2. When aqueous acids and bases are mixed together, what is the resultant pH?

The answer to both questions is YES!!! Let's work with the first question first
(and the second question second). Suppose we have a solid acid. We know that it's an
acid, and we know that it's pure, but we don't know anything else about it. We can
*titrate* this acid with base of known concentration to get information about the acid.

Suppose we weigh 2.00 g of the acid and suspend this in water. We check the pH
of the solution, and the pH is low, say 2.00. If we add 1.0 N sodium hydroxide to this
solution (is everyone OK in discussing *normality* relative to *molarity*?) in small
increments, say 0.5 ml, and measure the pH after every addition, the curve
representing the pH versus moles of hydroxide ion added may look something like one
of these three cases:

1. Strong monoprotic acid. An example of a strong monoprotic acid is HCl. As base is
   added, the $H^+$ in solution are sucked up, with small changes in pH, until almost all of
   the protons are consumed. When the concentration of protons equals the
   concentration of hydroxide ions, the solution pH is 7. Adding a little more hydroxide
   results in a big excess of hydroxide, and therefore a high pH.
2. Weak monoprotic acid. The second titration curve is typical of a monoprotic weak acid. At the beginning of the titration, there is a high concentration of free protons in solution, since the only proton acceptor in solution is the conjugate base of the acid. As hydroxide ions are added, some of the protons in solution add to the hydroxide ions, forming water. The equilibrium between the conjugate acid base pair then adjusts as the proton ion concentration decreases. When the concentration of the conjugate acid is equal to the concentration of the conjugate base, then the two cancel from the equilibrium expression and

\[ K_a = [H^+] \quad \text{or} \quad pK_a = pH \]

Under the conditions of the titration, this point is reached when half of the number of moles of hydroxide ions are added relative to the initial concentration of the acid. Graphically, this shows up as an inflection point on the graph, where the inflection point represents the point in the curve where the sign of the slope changes. The inflection point is more obvious when you take the first derivative of this curve, where the change in sign in slope will appear as a sharp peak. Knowing the inflection point will give you the pK\textsubscript{a} of the weak acid. Knowing the number of moles of hydroxide added to reach this inflection point will give you the number of moles of acid in solution.

Since we recorded the initial weight of acid added to solution (2.0 g), the gram molecular weight of the compound can be determined.

3. Polyprotic weak acid. Let's move to the third titration curve which is a more complicated case. Here, there are three different inflection points. This means that this weak acid contains three titratable groups. The removal of protons from these three ionizable groups are characterized by three different pK\textsubscript{a} values. In the above case, we can treat the three different protonations separately from one another. As we will see with amino acids, sometimes the titration of one ionizable group will overlap with the titration of a second ionizable group on the same molecule.
BUFFERS AND BUFFERING CAPACITY

An acid/base conjugate pair in aqueous solution, such as benzoic acid and benzoate ion, gives the solution the ability to resist changes in pH when acids or bases are added. This is demonstrated using the titration curves we just described. On this curve, we are adding linear increments of base. At some parts of the curve, the change in pH upon adding a small amount of base is large. At other parts of the curve, adding base results in little change in the solution pH. By inspection, we see that the conjugate acid/base pair resists changes in pH best, or has its highest buffering capacity, when the pH of the solution is near the pKₐ of the conjugate acid.

We can demonstrate this concept in a more mathematical fashion. Remember the expression for the Kₐ of an acid:

\[ K_a = [H^+] [A^-] / [HA] \]

Rearranging:

\[ 1 / [H^+] = (1 / K_a) ([A^-] / [HA]) \]

Take the logarithm of both sides of the equation

\[ \log (1 / [H^+]) = \log (1 / K_a) + \log ([A^-] / [HA]) \]

Substitute pH and pKₐ into this expression yields the Henderson-Hasselbach equation:

\[ pH = pK_a + \log ([A^-] / [HA]) \]

Let me quickly demonstrate the concept of solution buffering. Suppose that
you take 1 liter pure distilled water and add 1 ml of 10N HCl to this. What will the final pH be? If we ignore the change in volume for the sake of ease of calculation, we have added 0.01 moles of H\(^+\) to the water, making the concentration 0.01 M. If you take the negative log of the concentration, you end up with a pH of 2.

Next, suppose that you add to 1 liter of water 0.1 moles of benzoic acid and 0.1 moles of sodium benzoate. What will the pH of this solution be? Here, HA = benzoic acid and A\(^-\) = sodium benzoate. The concentrations of both compounds is 0.1 M. Therefore, on substitution, the log of 1 is 0, and the pH is equal to the pK\(_a\) of benzoic acid, which is 4.2.

Now, suppose you now add 1 ml of 10 N HCl to the benzoic acid solution. What will the pH be? We'll ignore the change in volume, since 1 ml is not significant to 1 liter. We've added .01 moles of [H\(^+\)] to the solution, which will add to 0.01 moles of benzoate to form benzoic acid. Therefore, the concentrations of benzoate ion is now 0.09 M, and the concentration of benzoic acid is 0.11 M. Substituting into the Henderson-Hasselbach equation yields.

\[
pH = 4.2 + \log \left( \frac{0.09}{0.11} \right) = 4.2 + \log (0.818)
\]

\[
= 4.2 + (-0.087) = 4.11
\]

Thus, the change in pH is 0.09 units for the addition of 0.01 moles of acid.

Several things to remember about buffers.

1. The buffering capacity of a buffer is greatest when the pH of the solution is around the pK for the conjugate acid/base pair. In practice, if we want to buffer a solution at a certain pH, a buffer is chosen such that the pK of the buffer is preferentially within 0.5 units from the desired pH (and certainly no more than 1 pH unit).

2. The higher the concentration of the conjugate acid/base pair, the better the buffering capacity of the solution.