Welcome to our Biochemistry text. We’re glad you’re here. In this chapter we introduce the subject and talk about the scientific aspects of the most important and most abundant liquid on the face of the Earth - water.
Introduction

Welcome to Biochemistry Free and Easy. As biochemistry instructors, we are always delighted when anyone shows an interest in our favorite subject. Helping students understand and enjoy biochemistry is our motivation for writing this book. It is not our intention to provide a comprehensive account of the chemical basis of life. Instead, we have tried to help students understand this fascinating subject by focusing on some key topics and concepts. This pared-down approach can be helpful for novices who might otherwise lose sight of important organizing principles in a sea of detail. The electronic format has also allowed us to provide multimedia links, in the form of video lectures and biochemistry songs to help students learn the subject. Best of all, this format makes it practical for us to distribute the book at no cost to anyone who wishes to learn basic biochemistry.

Biochemistry is a relatively young science, but the rate of its expansion has been truly impressive. This rapid pace of discoveries, which shows no signs of slowing, is reflected in the steady increase in the size of biochemistry textbooks, most of which top a thousand pages and undergo revisions every couple of years to incorporate new...
findings. These full-scale texts offer an enormous amount of information and serve as invaluable resources. Those who need the greater level of detail and broader coverage that these books provide have many choices available in any good bookstore.

As certified (some might say, certifiable) biochemistry nerds and unrepentant lovers of corny jokes, we firmly believe that students can have fun while learning the subject. Toward this end, we have sprinkled each chapter with rhymes and songs that we hope will have you learning biochemistry happily. The format of the book as available for the iPad, allows readers to click on figures to enlarge them, watch video lectures relevant to each topic, listen to the songs in the book, like the one above, and link out to the internet to find more information.

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**Biochemistry, Biochemistry**

To the tune of “Oh Christmas Tree”

Biochemistry Biochemistry
I wish that I were wiser
I feel I’m in way o’er my head
I need a new advisor

Biochemistry Biochemistry
My courses really shouldn’t be
Such metabolic misery
Biochemistry Biochemistry
I wish that I were wiser

Biochemistry Biochemistry
Reactions make me shiver
They’re in my heart and in my lungs
They’re even in my liver

I promise I would not complain
If I could store them in my brain
Biochemistry Biochemistry
I wish that I were wiser

Biochemistry Biochemistry
I’m truly in a panic
Your mechanisms murder me
Biochemistry Biochemistry
I should have learned organic

For all I have to memorize
I ought to win the Nobel Prize.
Biochemistry Biochemistry
I wish that I were wiser

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Recorded by Tim Karplus.
Lyrics by Kevin Ahern
simply by clicking on any term. If you are using a PDF version of this book, you will still be able to use the links to the video lectures. Also, though you cannot listen to the songs by clicking on them in the PDF version, you can download them HERE. We hope you find these features useful and that they help you learn biochemistry.

**Cells: The Bio of Biochemistry**

Biochemistry happens inside organisms and possibly, the most obvious thing about living organisms is their astounding diversity. If living things are so varied, it seems reasonable to ask whether their chemistry is, too.

The invention of the microscope opened up a whole new world of microscopic organisms while also providing the first clue that living organisms had something in common—all living things are made up of cells. Some cells are “lone rangers” in the form of unicellular entities, such as bacteria and some protists. Cells are also the building blocks of more complex organisms (like humans, wombats, and turnips).

As increasingly powerful microscopes became available, it was possible to discern that all cells fell into one of two types—those with a nucleus and other sub-cellular compartments like mitochondria and lysosomes, termed eukaryotes, and those that lack such internal compartmentation, the prokaryotes. Some eukaryotes, such as yeast, are unicellular, while others, including animals and plants are multicellular. The prokaryotes may be divided into two very broad categories, the bacteria and the archaeans.

One can find living cells almost everywhere on earth—in thermal vents on the ocean floor, on the surface of your tongue and even in the frozen wastes of the Antarctic. Some cells may
have even survived over two years on the moon. Yet, despite their diversity of appearance, habitat, and genetic composition, cells are not as different from each other as you might expect. At the biochemical level, it turns out that all cells are more alike than they are different. A great simplifying feature of biochemistry is that many of the reactions are universal, occurring in all cells. For example, most bacteria process glucose in the same 10-step pathway that plant, animal, and fungal cells do. The genetic code that specifies the amino acids encoded by a nucleic acid sequence is interpreted almost identically by all living cells, as well. Thus, the biochemical spectrum of life is (mercifully) not nearly as broad or as complicated as the evolutionary spectrum. Where cells differ significantly in processes/reactions, we will note these differences.

**Water, Water, Everywhere**

Vital for life, water is by far the most abundant component of every cell. To understand life, we must, therefore, understand the basics of water, because everything that happens in cells, even reactions buried deep inside enzymes, away from water, is influenced by water's chemistry.
We start with simple properties. The molecule has a sort of wide ‘V’ shape (the H-O-H angle is 104°) with uneven sharing of electrons between the oxygen and the hydrogens. The hydrogens, as a result, are described as having a partial positive charge and the oxygen has a partial negative charge. These tiny partial charges allow the formation of what are described as hydrogen bonds, which occur when the partial positive charge of one atom is attracted to the partial negative of another. In water, that means the hydrogen of one water molecule will be attracted to the oxygen of another. Hydrogen bonds play essential roles in proteins, DNA, and RNA, as well, as we shall see.

**Buffers Keep the Cellular Environment Stable**

Water can ionize to a slight extent ($10^{-7}$ M - about 6 molecules per 100 million of pure water) to form H$^+$ (proton) and OH$^-$ (hydroxide). We measure the proton concentration of a solution with pH, which we define as the negative log of the proton concentration.

\[
pH = -\log[H^+]
\]

If the proton concentration, $[H^+] = 10^{-7}$ M, then the pH is 7. We could just as easily measure the hydroxide concentration with the pOH by the parallel equation,

\[
pOH = -\log[OH^-]
\]

In pure water, dissociation of a proton from it creates a hydroxide, so the pOH of pure water is 7, as well. This also means that

\[pH + pOH = 14\]

Why do we care about pH? Because biological molecules can, in some cases, be exquisitely sensitive to changes in it. As the pH of a solution changes, the charges of molecules in the solution can change, as you will see. Changing charges on biological molecules, especially proteins, can drastically affect how they work and even whether they work at all.
Because protons and hydroxides can combine to form water, a large amount of one will cause there to be a small amount of the other. Why is this the case? In simple terms, if I dump 0.1 moles of $H^+$ into a pure water solution, the high proton concentration will react with the relatively small amount of hydroxides to create water, thus reducing hydroxides. Similarly, if I dump excess hydroxide (as NaOH, for example) into pure water, the proton concentration falls for the same reason.

Chemists use the term “acid” to refer to a substance which has protons that can dissociate (come off) when dissolved in water. They use the term “base” to refer to a substance that can absorb protons when dissolved in water. Both acids and bases come in strong and weak forms. (Examples of weak acids are shown on the previous page.)

Strong acids, such as HCl, dissociate completely in water. If we add 0.1 moles of HCl to a solution to make a liter, it will have 0.1 moles of $H^+$ and 0.1 moles of $Cl^-$. There will be no remaining HCl when this happens. A strong base like NaOH also dissociates completely into Na$^+$ and OH$^-$. Weak acids and bases differ from their strong counterparts. When you put one mole of acetic acid (HAc) into pure water, only about 4 in 1000 HAc molecules dissociate into $H^+$ and Ac$^-$. Thus, if I start with 1000 HAc, I will end up with 996 HAc and 4 each of $H^+$ and Ac$^-$. Clearly, weak acids are very different from strong acids. Weak bases behave similarly, except that they accept protons, rather than donate them.
You may wonder why we care about weak acids. You may never have thought much of weak acids when you were in General Chemistry. Your instructor described them as buffers and you probably dutifully memorized the fact that “buffers are substances that resist change in pH” without really learning what it meant. We will not allow that to happen here.

Weak acids are critical for life because their affinity for protons causes them to behave like a UPS. We’re not referring to the UPS that is the United Parcel Service®, but instead, to the encased battery backup systems for computers called Uninterruptible Power Supplies that kick on to keep a computer running during a power failure. Your laptop battery is a UPS, for example. We can think of weak acids as Uninterruptible Proton Suppliers within certain pH ranges, providing (or absorbing) protons as needed. Weak acids thus help to keep the H⁺ concentration (and thus the pH) of the solution they are in relatively constant.

Consider the acetic acid (acetate) system. Here is what happens when HAc dissociates

\[ \text{HAc} \leftrightarrow \text{H}^+ + \text{Ac}^- \]

As noted, about 4 in 1000 HAc molecules come apart. However, what if one started adding hydroxyl ions (by adding a strong base like NaOH) to the solution with the HAc in it? As the added OH⁻ ions reacted with the H⁺ ions to make water, the concentration of H⁺ ions would go down and the pH would go up. However, in contrast to the situation with a solution of pure water, there is a backup source of H⁺ available in the form of HAc. Here is where the UPS function kicks in. As protons are taken away by the added hydroxyl ions (making water), they are partly replaced by protons from the HAc. This is why a weak acid is a buffer. It resists changes in pH by releasing protons to compensate for those “used up” in reacting with the hydroxyl ions.

**Henderson-Hasselbalch**

It is useful to be able to predict the response of the HAc system to changes in H⁺ concentration. The Henderson-Hasselbalch equation defines the relationship between pH and the ratio of Ac⁻ and HAc. It is as follows

\[ \text{pH} = \text{pK}_a + \log \left( \frac{[\text{Ac}^-]}{[\text{HAc}]} \right) \]

This simple equation defines the relationship between the pH of a solution and the ratio of Ac⁻ and HAc.
and the ratio of Ac⁻ and HAc in it. The new term, called the pKa, is defined as

\[ pK_a = -\log K_a, \text{ just as } pH = -\log [H^+] \]

The \( K_a \) is the acid dissociation constant and is a measure of the strength of an acid. For a general acid, HA, which dissociates as

\[ HA \leftrightarrow H^+ + A^-, \]

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

Thus, the stronger the acid, the more protons that will dissociate from it and the larger the value its \( K_a \) will have. Large values of \( K_a \) translate to lower values of pKa. As a result, the lower the pKa value is for a given acid, the stronger the acid is.

Please note that pKa is a constant for a given acid. The pKa for acetic acid is 4.76. By comparison, the pKa for formic acid is 3.75. Formic acid is therefore a stronger acid than acetic acid. A stronger acid will have more protons dissociated at a given pH than a weaker acid.

Now, how does this translate into stabilizing pH? The previous figure shows a titration curve. In this curve, the titration begins with the conditions at the lower left (very low pH). At a this pH, the HAc form predominates, but as more and more OH⁻ is added (moving to the right), the pH goes up, the amount of Ac⁻ goes up and (correspondingly), the amount of HAc goes down. Notice that the curve “flattens” near the pKa (4.76). What this tells us is that the pH is not changing much (not going up as fast) as it did earlier when the same amount of hydroxide was added. The system is resisting a change in pH (not stopping...
the change, but slowing it) in the region of about one pH unit above and one pH unit below the pKa. Thus, the buffering region of the acetic acid/acetate buffer is from about 3.76 to 5.76. It is maximally strong at a pH of 4.76.

Now it starts to become apparent how the buffer works. HA can donate protons when extras are needed (such as when OH⁻ is added to the solution. Similarly, A⁻ can accept protons when extra H⁺ are added to the solution (adding HCl, for example). The maximum ability to donate or accept protons comes when

\[ [A^-] = [HA] \]

To understand how well a buffer protects against changes in pH, consider the effect of adding .01 moles of HCl to 1.0 liter of pure water (no volume change) at pH 7, compared to adding it to 1.0 liter of a 1M acetate buffer at pH 4.76. Since HCl completely dissociates, in 0.01M (10⁻² M) HCl you will have 0.01M H⁺. For the pure water, the pH drops from 7.0 down to 2.0 (pH = -log(0.01M)).

By contrast, the acetate buffer’s pH is 4.74. Thus, the pure water solution sees its pH fall from 7 to 2 (5 pH units), whereas the buffered solution saw its pH drop from 4.76 to 4.74 (0.02 pH units). Clearly, the buffer minimizes the impact of the added protons compared to the pure water.

It is important to note that buffers have capacities limited by their concentration. Let’s imagine that in the previous paragraph, we had added the 0.01 moles HCl to an acetate buffer that

<table>
<thead>
<tr>
<th>pH</th>
<th>[Salt]/[Acid] Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>pKa + 3</td>
<td>1000</td>
</tr>
<tr>
<td>pKa + 2</td>
<td>100</td>
</tr>
<tr>
<td>pKa + 1</td>
<td>10</td>
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<tr>
<td>pKa +</td>
<td>1</td>
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<tr>
<td>pKa - 1</td>
<td>1/10</td>
</tr>
<tr>
<td>pKa - 2</td>
<td>1/100</td>
</tr>
<tr>
<td>pKa - 3</td>
<td>1/1000</td>
</tr>
</tbody>
</table>

Why do we care about buffers? Buffers help to keep the pH of a solution from changing much, even when protons are added to it or removed from it. When you exercise, your muscles produce protons, which get dumped into the blood. If a buffer were not present, the pH of the blood would change drastically and you would likely die, since the acidification of your blood would denature/inactivate most of your enzymes.

Why does the pKa give the pH of the maximum buffering capacity? From the Henderson-Hasselbalch equation, when pH = pKa, the log term (\( \log([A^-]/[HA]) \)) must be zero. For the log term to be zero, \([A^-]\) must equal \([HA]\).
Henderson Hasselbalch
To the tune of "My Country 'Tis of Thee"

Henderson Hasselbalch
You put my brain in shock
Oh woe is me
The pKa's can make
Me lie in bed awake
They give me really bad headaches
Oh hear my plea
Salt - acid RA-ti-os
Help keep the pH froze
By buf-fer-ING
They show tenacity
Complete audacity
If used within capacity
To maintain things
I know when H’s fly
A buffer will defy
Them actively
Those protons cannot waltz
When they get bound to salts
With this the change in pH halts
All praise to thee
Thus now that I’ve addressed
This topic for the test
I’ve got know-how
The pH I can say
Equals the pKa
In sum with log of S o’er A
I know it now

concentration of 0.01M and equal amounts of Ac⁻ and HAc. When we try to do the math in parallel to the previous calculation, we see that there are 0.01M protons, but only 0.005M A⁻ to absorb them. We could imagine that 0.005M of the protons would be absorbed, but that would still leave 0.005M of protons unbuffered. Thus, the pH of this solution would be approximately

$$\text{pH} = -\log(0.005\text{M}) = 2.30$$

Exceeding buffering capacity dropped the pH significantly compared to adding the same amount of protons to a 1M acetate buffer. Consequently, when considering buffers, it is important to recognize that their concentration sets their limits. Another limit is the pH range in which one hopes to control proton concentration.

Now, what happens if a molecule has two (or more) ionizable groups? It turns out, not surprisingly, that each group will have its own pKa and, as a consequence, will tend to ionize at different pH values. The figure above right shows the titration curve for a simple amino acid, alanine. Note that instead of a single flattening of the curve, as was seen for acetic acid, alanine displays two such regions. These are individual buffering regions, each centered on the respective pKa values for the carboxyl group and the amino group.

Recorded by David Simmons
Lyrics by Kevin Ahern and Indira Rajagopal
If we think about alanine, it can have three possible charges: +1 (alpha carboxyl group and alpha amino group each has a proton), 0 (alpha carboxyl group missing a proton and alpha amino group has a proton) and -1 (alpha carboxyl group and alpha amino group each lacking a proton).

How does one predict the charge at a given pH for an amino acid? A good rule of thumb for estimating charge is that if the pH is more than one unit below the pKa for a group (carboxyl or amino), the proton is on. If the pH is more than one unit above the pKa for the group, the proton is off. If the pH is NOT more than one or less than one pH unit from the pKa, this simple assumption will not work. Further, it is important to recognize that these rules of thumb are estimates only. The pI (pH at which the charge of a molecule is zero) is an exact value calculated as the average of the two pKa values around the point where the charge of the molecule is zero.

Reference
Around the Nucleus
To the tune of “Across the Universe”

DNA gets spooled like balls of yarn
Within the chromosomes
Unwinding when it’s duplicated there
Around the nucleus

Primase sets down RNA
To pave the path for DNA
Across a replication fork

Complementarity rules (ahhhhh)
DNA polymerase
Synthesizing DNAs
RNA polymerase
Making all the RNAs

Helicases split the strands
In front of replication forks
To make templates accessible
Around the nucleus

Complementary bases
Match the bonds of ‘H’ and hold the strands
Together till they’re pulled apart

Around the nucleus
Hydrogen bonding fuels (ahhhhhhhhh)
Tiny alpha helix bands
Folding for the cells’ demands
Beta sheets comprised of strands
Meeting all the cells’ demands

Exons link majestically all guided
By a master plan encoded in the cell’s genome
That’s buried deep inside of me
Countless combinations of the codons
Bring diversity to life evolving on and on
Around the nucleus

Complexes rule the world (ahhhhhhhhh)
Ribosomes and spliceosomes
Transforming the cells’ genomes
Ribosomes and spliceosomes
Builders of the proteomes

YouTube video HERE

Recorded by David Simmons
 Lyrics by Kevin Ahern