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Characteristics of Enzymes

At university, Enzymology was the class that most biochemistry or biology majors dreaded taking. Those students who liked the class were typically math majors who took the class for the thrill of solving complex rate equation derivations. Those students who had to take the class against their will were those who might need to understand the role of enzymes as they pertained to other aspects of biochemistry, but otherwise had little desire to sit through boring lectures involving lots of equations and the occasional molecular structures on the white board. As a professor teaching biochemistry to undergraduate and graduate students the task fell to me to keep my students' attention, so they didn't fall asleep, yet challenge them to understand why what I was teaching them could be both fun and useful.

I started teaching traditional enzymology as it was presented in the textbooks of the day (and I'm sorry to say is still being presented today). I found my students were passing the tests, but failing to understand how to interpret data and more importantly how to fit the data they were obtaining in their research into something meaningful and exciting. I eventually adopted a strategy of engaging the minds of my students with challenging, but improbable, enzymatic mechanisms and found that steady state kinetics, while more relevant, hindered the understanding of some of the more basic principles associated with enzyme kinetics. I finally hit upon the use of quasi-equilibrium assumptions and my students began to question and challenge my lectures—I had finally arrived as a professor.

Enzymology can be the study of enzymes as protein molecules with specific folding patterns of the amino acid polymer and unique binding sites wherein intra- and inter-molecular distances define the specificity of the molecule to attract, bind to, and change some substrate molecule. Computer-aided molecular modeling is a wonderful aspect of both biochemistry and enzymology in providing visuals essential to understanding, but does little to help with data analysis. Alternatively, enzymology can be the study of how these protein molecules control and mediate the flow of metabolites through intermediary metabolism affecting what we call metabolic viability to life forms. It is this latter study of enzymes that will be the focus of this book.

Enzymes are mostly proteins that are of variable length (with respect to amino acid sequences) and molecular weight. These amino acid polymers typically fold into some conformation that is most energetically favored based on the nature of

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the amino acids making up the protein and the aqueous environment in which they find themselves. For the most part, hydrophobic amino acids such as leucine or phenylalanine, as examples, are to be found in what might be called the hydrophobic core of the protein, whereas the hydrophilic amino acids such as histidine or aspartic acid, again as examples, will preferentially be found on those surfaces of the protein more exposed to an aqueous (hydrophilic) environment. The arrangement of hydrophobic and ionizable side groups of these hydrophilic amino acids is typically described as being present in some molecular organization that forms a region complementary to some low molecular weight solute. This region of the protein is generally regarded as constituting the substrate- (or modifier-) binding site. Whether this binding site tends to bind an unstable form of the substrate, stabilizing the unstable intermediate, and in so doing promoting its conversion to product; or whether this binding site tends to bind a stable form of the substrate and in so doing causes the substrate to shift into some less stable configuration promoting its conversion to product, will be discussed in detail. We will enter into this aspect of the basics of enzymology in detail in Chapter 2. For now, I only wish to stipulate that this enzyme with a substrate-binding site will be referred to as free enzyme (E) in subsequent sections. When free enzyme (E) binds with the substrate, it will be referred to as the enzyme/substrate complex (ES). As the enzyme facilitates the conversion of substrate to product via some unknown or unspecified mechanism, the product (P) released from the substrate-binding site will result in the (ES) complex reverting to free enzyme (E). Thus within the context of this book, the sum of the concentration of free enzyme (E) and enzyme/substrate complex (ES) will be referred to as the total amount (or quantity) of enzyme (E_t). When introducing modifiers of enzyme activity, I will use the simple connotation of a modifier (M) being either an activator (Ma) or an inhibitor (Mi). Modifiers will typically bind to free enzyme (E) to form a modified enzyme as either (MaE) or (MiE). Where substrate (S), enzyme (E), substrate/enzyme complex (ES), and so forth, are bracketed with square brackets, such as [S], the intent will be to express the molecule as some concentration. I will try to restate this point throughout the text, more to remind and help you than to irritate you with what will appear as my being overly redundant. Repetition is a good learning tool.

I would also like to emphasize one more point. I will make reference to "saturating" concentrations of substrate or modifier in the text. As you will see in later figures, as you add increasing concentrations of substrate (or modifier) to an enzymatic reaction, the rate of conversion of substrate to product will gradually increase until such time as that concentration approaches the capacity of that enzyme to bind to substrate converting it to product. At such a time where increasing the concentration of substrate no longer significantly increases the rate of conversion to product, it is generally assumed (described) as a saturation of enzyme by substrate. This will make more sense later, but I also want to emphasize that we will operate under the premise that the amount of substrate at any given concentration of that substrate will be inexhaustible. This means basically that you can crystallize salt out of sea water, but you will never run out of sea water where there is an infinite amount of salt. This is the difference between the concentration of salt in sea water and the amount of salt in the sea.

I shall take a rather simplistic approach to the overall mathematical equation subject with respect to enzymes by defining a few selected terms. As you get deeper into the study of enzymes and enzymology you will have an opportunity to learn that in seeking generalities, one must frequently stretch the truth a bit in order to understand the "why" when it comes to enzymes as mediators of intermediary metabolism. I will work almost exclusively under what is generally referred to as quasi-equilibrium assumptions, rather than the more probable and ultimately more useful steady-state assumptions to describe enzyme kinetic mechanisms and associated rate constants. Later on in your studies, you can move onto steady-state assumptions, but for now I will take a bit of poetic license and work under quasi-equilibrium assumptions.

Thermodynamics

For now, let's think about the role of an enzyme and what we need to think about when it comes to an enzyme performing that role. Enzymes, as proteins in solution, have three simplistic energies. They have vibrational energy, which is simply the tendency of atoms and groups of atoms to present energy dissipation or collection as more or less a degree of stability/instability without presenting as either of the two other forms of energy. They also have rotational energy, which is simply the tendency of a molecule (collection of atoms) to "roll" or "spin" in place when in "solution." Finally, they have translational energy, which is simply the tendency of a molecule to move in some direction until events cause it to change that direction in favor of a second direction. Temperature has an impact on all three forms of energy in an enzyme, and we shall attempt to cover how all three forms of energy in an enzyme (as well as their substrates) factor into the role of an enzyme in speeding the rate of conversion of substrate to product without being consumed in the reaction. However, as I stated above, we will get more into this topic in Chapter 2. This chapter has more to do with trying to define terms than trying to explain how they help in describing how an enzyme functions.

Temperature has an obvious role in enzyme activities and a very complex role. Temperature changes directly impact on the vibrational energy of molecules such as substrates of enzymatic reactions. Using the brief description of vibrational energy in the previous paragraph, it is easy to suggest that as the temperature in which a substrate molecule (as well as an enzyme, but let's leave the enzyme out for now) finds itself, the increased vibrational energy will tend to present as increased movement of atoms relative to their covalent bonds, as movements of electrons in possible orbits around their nuclei, and/or as overall changes in the structural conformation of the molecule (substrate in this instance). In some respects, increases in vibrational energy may represent the more significant aspect of what has been described as the "energy of activation" of some molecule necessary for that molecule to undergo a spontaneous chemical reaction becoming another molecule (perhaps a "product" for sake of my keeping in focus with this book). As a molecule becomes "activated" through the introduction of energy—in the form of increased temperature(s)—more of the

substrate molecules will possess sufficient energy to acquire that "energy of activation"; and since the spontaneous chemical reaction will be defined as a concentration times some rate constant, the higher concentration of "activated" (energized) substrate will result in a faster rate of chemical change of that substrate into a product. You will encounter this issue again in Chapter 2 and Figure 2.4. However, this is a book about enzymes, and I would be remiss if I left the rather loose definition of "energy of activation" to apply only to "vibrational energy" of a molecule (substrate). Temperature also has effects on rotational and translational energies of molecules involved in some enzymatic reaction. As temperatures increase in some enzymatic reaction, molecules will tend to rotate and translate more freely, and while such rotational and translational energies may have less to do with the "energy of activation" component of a spontaneous chemical reaction, they most likely have more of an effect on the "energy of activation" associated with the enzyme-driven spontaneous chemical reaction than vibrational energy. So, how to define "energy of activation" for how we wish it to be used in the context of this book? The first thing we have to understand is that the energy of activation of a molecule that will undergo a spontaneous chemical reaction is not the same energy of activation of a molecule that will undergo a spontaneous chemical reaction where the rate of that spontaneous chemical reaction is enhanced through mediation of an enzyme (catalyst?). The energy of activation of the latter reaction should thus include roles for temperature, solution effects, enzyme, substrate, vibrational energies, rotational energies, and translational energies (note the use of "energies" here in that both the substrate and the enzyme possess these characteristics).

Like temperature, the solution in which an enzyme (and its substrates) is dissolved has an impact on all three forms of energy in an enzyme, but this impact is far more complicated than the role of temperature, although as we will see later, temperature has considerable impact on the nature of the solution and how that nature of the solution bears on the three forms of energy and subsequent interactions of the enzyme with its substrate(s) (and formed products). Enzymes function naturally in an aqueous solution of water and various ions (and/or other solutes). Water consists of a unique molecule consisting of one oxygen and two hydrogen atoms. The hydrogen atoms are situated on one side of the oxygen atom, and both kinds of atoms share electrons such that the electrons tend to spend more time with the oxygen atom than with the corresponding hydrogen atom(s) giving the whole molecule a dipole moment. This dipole moment imparts a slight negative nature to the oxygen side of the water molecule and a slight positive nature to the hydrogen side of the water molecule. It is this dipole moment that gives the water solution characteristics relevant to the energies of the enzyme, the energies of the substrate (and formed product), and directly impacts on the energy of activation and the kinetics of the enzymatic reaction in the conversion of substrate to product by that enzyme.

Normally water molecules are oriented rather randomly (with respect to their dipole moments) in some aqueous solution. However, lowering the temperature begins to remove energy from those water molecules, and at some sufficiently low temperature the water molecules will begin to lose rotational and translational energy and begin to align themselves according to their

dipole moment such that the more positively charged side will be attracted to the more negatively charged side of a second molecule; eventually the water molecules will assume a "crystalline-like" structure where the dipole moments of the water molecules will all be oriented in mostly the same direction. This crystalline-like (or paracrystalline) form of water is called ice; ice presents a solution of water molecules with a lower entropy (less fluidized) than a solution of water molecules randomly associated as in a more fluidized or "liquid" solution state.

I will avoid the topic of thermodynamics, but it is important to introduce you, at this point, to a very simple equation that I think will help you to understand how the energies of an enzyme (and/or its substrate) and the solution in which it is dissolved impact the role of that enzyme. Equation 1.1 is pretty simple and describes how we currently relate entropy, enthalpy, and free energy.

$$\Delta G = \Delta H - T\Delta S \tag{1.1}$$

The symbol Δ (delta) refers to change, *G* represents free energy, *H* represents enthalpy, *T* represents temperature (in degrees Kelvin), and *S* represents entropy. Thus, in simple terms, the change in free energy of a system equals the change in enthalpy minus the change in entropy (times the temperature). In general, if the change in enthalpy is positive the change in free energy is positive. In general, if the change in entropy is positive (no change in temperature) the change in free energy is negative. Conversely, if the change in entropy is negative (and enthalpy is constant), the system gains energy and the change in free energy is positive. If you wish to relate these events to something you are more familiar with, remember that in the universe, all the other galaxies are moving away from our galaxy as fast as they can (a large negative delta *S*, or an increase in entropy for the universe). I will leave you to speculate whether these other galaxies know something we don't know or whether it is this increase in entropy that facilitates the condensation of matter into a galaxy (the negative delta *G* for this process?). These relationships between entropy and enthalpy pertain, at least for purposes of this book, to whether or not a chemical reaction will be spontaneous or nonspontaneous, that is, whether the chemical reaction to be catalyzed by some enzyme will result in the "release" of energy (spontaneous) or require the "input" of energy (nonspontaneous) for the reaction to proceed. As a general rule, if the ΔG is positive, the chemical reaction will not be spontaneous and if the ΔG is negative, the chemical reaction will be spontaneous. The roles of ΔH (enthalpy) and ΔS (entropy) in determining whether or not some chemical reactions (and thus the enzyme catalysis of the chemical reactions) are best illustrated in Table 1.1. It is good to remember that a spontaneous chemical reaction can proceed slowly (over a very long time) or very quickly (over a very short period of time—as in a chemical explosion) and some spontaneous chemical reactions may occur so slowly as not to be measurable in the time frame under consideration. Thus in some cases a spontaneous chemical reaction may appear to be nonspontaneous and one should be careful in predicting whether or not an enzyme will actually speed the rate of some chemical reaction.

Table 1.1 The relationships between entropy and enthalpy in determining whether or not some chemical reaction will be spontaneous or nonspontaneous.

Enthalpy (ΔH)	Entropy (ΔS)	Chemical reaction is:	Relevant conditions	Comments
Positive	Negative	Nonspontaneous	Always	
Negative	Positive	Spontaneous	Always	
Negative	Negative	Nonspontaneous	Can be spontaneous	Spontaneous if temperature (T) is lowered
Positive	Positive	Nonspontaneous	Can be spontaneous	Nonspontaneous involves absolute values of ΔH and $T\Delta S$, and ΔH is greater than $T\Delta S$ Can be made spontaneous by raising temperature (T)

Now, we normally associate entropy with the degree of disorder in some system, and thus we would consider that water in a crystalline state (ice) is more ordered than water not in a crystalline state and thus this increase in order represents a solution with less entropy. It is important to understand that entropy and enthalpy in this equation are not independent when it comes to changes in free energy, but the objectives here are to try to illustrate how a water solution with more order (less entropy) will impede the "rotational" and "translational" energies of an enzyme (and its substrate) because for the enzyme to move through a solution of water molecules possessing some degree of order so as to interact with its substrate, that enzyme (and/or its substrate) will have to destabilize that ordered structure of the water solution (effectively contribute to a localized increase in entropy for the water molecules). And the only way that water can be destabilized is by contributing to a decrease in free energy, for example a negative delta *G* (*H* and *T* of course being held constant) or mitigation of their dipolar moments, which pretty much amounts to the same thing. This is not a difficult concept to grasp, but it is a difficult concept to present without getting deeper into thermodynamics. Suffice it to say that in examining the role of an enzyme in changing some substrate to some product it is important to understand that the nature of the water molecules in which that enzyme (and substrate) are dissolved is important. A solution of water that is more ordered will require the input of energy to make it less ordered (or more disordered) so that the enzyme and substrate can more easily approach each other and bind to permit the catalytic event converting substrate to product.

It is not just temperature that impacts on the order/disorder of the water solution in which an enzyme (and/or substrate) is dissolved. Charged atoms such as sodium (Na^+) or chloride (Cl^-) aid in the mitigation of the dipole moment of water molecules through "charge neutralization." Thus, water containing simple saline will tend to be more fluidized (more disordered) at any temperature than water not containing saline because the salt ions will tend to minimize the value of the dipole moment of a water molecule in alignment of water molecules (less disordered) according to the charge distribution on the water molecule(s). However, charged groups on an enzyme (or a substrate) may tend to organize (stabilize) the structure of the water molecules in close proximity to charged groups (especially in the absence of dipole moment mitigation by ionizable salts such as sodium chloride) on that enzyme (or substrate). Since water molecules have that dipole moment, positively charged groups on the enzyme (or substrate) will tend to order the water molecule in one direction (with respect to the partial positive/negative charge distribution of the water molecules), and negatively charged groups will tend to order the water molecules in the opposite direction (again with respect to the partial positive/negative charge distribution of the water molecules) (see Figure 1.1). This more ordered water in close proximity to a charged group on an enzyme may be thought of as "bound water" (or ordered water). A loose analogy to this bound water might include some reference to a stream of water moving between two earthen banks (as with a "river"). Water in close proximity to the bank moves more slowly than water in the middle of the stream because of the stabilizing influence (i.e., frictional resistance) of the river bank. Water along the earthen bank possesses less energy than water in the

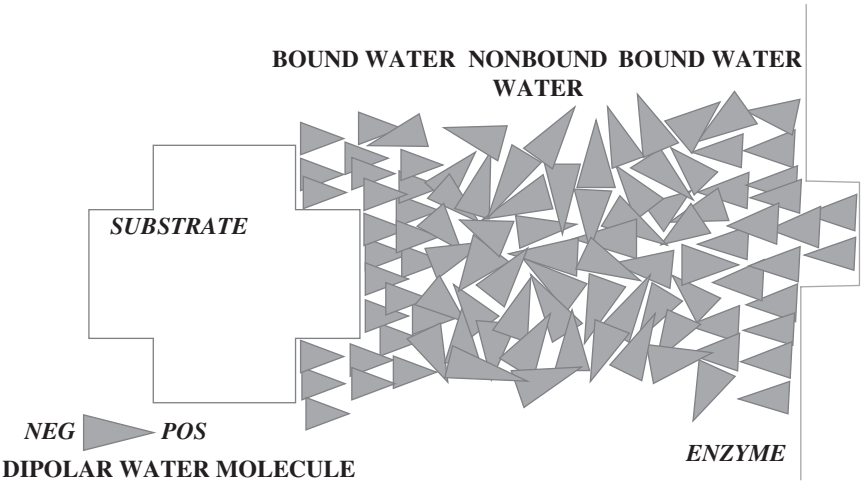

Figure 1.1 Illustration of the role of bound water versus nonbound water in the translational movement of a molecule of substrate into the substrate-binding site of an enzyme. (*See insert for color representation of the figure.*)

middle of the stream, and all things being equal is more ordered (i.e., has less entropy). The relevance of this to an enzyme-catalyzed reaction is, of course, that enzymes and substrates carry with them a layer of ordered water, and for the substrate to bind in some catalytic site on an enzyme the layers of ordered water must either participate in the binding reaction or must be disordered (increase in their entropies) for the substrate to bind in the catalytic site of the enzyme. It is easy to visualize (even if the characterization is incomplete in missing other aspects of the event) how a substrate with rotational and translational energy might give up some of that energy (less entropy for the enzyme/substrate complex) in exchange for disruption of the ordered water in some binding site facilitating binding of that substrate in the binding site. Perhaps you could imagine that the ordered (or bound) water at some enzyme binding site contributes to a reduction in the rotational (and translational) energy of the substrate as the substrate moves into the binding site and this action facilitates the binding? (Was that a good visual for the "gamers" amongst you?) If you visualized this point, you should begin to understand that binding of substrate (or modifier) in some binding site on an enzyme may be as much about the boundary water surrounding both molecules as it is about "markers" (charged or hydrophobic areas) on the substrate that the enzyme recognizes (and vice versa), and thus represent some aspect of the association "k_1" (and perhaps dissociation "k_{-1}") rate constants we will discuss in the next chapters.

We will get more into activity coefficients later in this chapter, but I would wish at this point to stipulate that with respect to activity coefficients of solutes (be they a substrate molecule or an enzyme molecule) size matters when it comes to translational and/or rotational energies. Larger molecules will present more bound water than smaller molecules, and you should therefore expect a substrate molecule to behave quite differently in solution than the enzyme molecule for a given concentration. Moreover, their concentrations (primarily increasing

concentrations) will behave in a progressively less and less ideal manner—meaning their activity coefficients may change from unity (or a value of 1) at low concentrations to less than unity at higher concentrations.

This point, of course leads to an additional point (definition?) to be made in this chapter. The point is that during the course of discussions in this textbook, I will always assume that the enzyme concentration present will be at such a dilute (lower) concentration, that the free enzyme (E) will always exist as a monomer (unless I otherwise stipulate aggregation events where proteins might tend to aggregate into dimers, trimers, etc.) and will therefore behave in a more ideal state.

A third variable impacting on the order/disorder of the water molecules in which an enzyme and its substrate are dissolved is the presence (or absence) of other solutes dissolved in the water molecules (the solvent). Such solutes can, and frequently do, include ionically charged salts (sodium ions, chloride ions, etc.), noncharged molecules such as sugars, or charged molecules such as amino acids. This is, of course, why water containing such solutes "freezes" at lower temperatures than water not containing such solutes—the solutes in a water solution get mixed into the slowly organizing molecules of water as the temperature drops and negatively impact on the formation of an organized structure (crystal) of water molecules. It is also why differing ionic solutions as buffers will import differing kinetics to enzyme-catalyzed chemical reactions. I provided a bit of discussion above regarding solutes such as sodium chloride and a possible role for this salt in mitigating the dipole moment of water, but what effect(s) might nonionically charged solutes, for example a simple sugar such as glucose, have on some enzyme-mediated chemical reaction? What about a more complex sugar such as a polysaccharide (noncharged of course)? Can you look at Figure 1.1 and imagine a role for a molecule much larger than a water molecule in impacting the order of water molecules both in the bulk solution and in the bound water molecules near the boundaries of the substrate and enzyme? What if a polysaccharide were to increase the viscosity of the aqueous solution?

Enough pseudo-thermodynamics; you should be getting the idea by now that in covering the roles of kinetic constants in rate equations, the roles of pH and ionic strength, as well as the roles of groups of amino acids in forming catalytic sites of enzymes, it will be important to be able to visualize in your mind that the interaction of a substrate in a catalytic site of an enzyme involves more than that substrate simply approaching and entering (in some proper orientation) some catalytic site for it to be converted to some other chemical form (i.e., a product). What happens when a substrate molecule enters some catalytic (binding) site with the "wrong" orientation necessary for the enzyme to bind and catalyze a chemical reaction? Does a substrate have some role as a "competitive inhibitor" when it binds improperly in the binding site restricting the binding of another substrate in that binding site in the proper orientation? I'm not about giving you the answers (as one reviewer early on asked about). I'm here to try to get you to think about what answers you can come up with. It is not about your (or my) answer being "right," it is about whether or not each of us will take the time and effort to think about the question. Did we even ask the right question?

Enzyme Nomenclature

I always found discussions of enzyme nomenclature to be very dry and to be that part of the course where most students quickly lost interest in my lecture. However, enzyme nomenclature is very important when it comes to understanding the differences between enzymes, and perhaps more importantly the information is something that can be easily remembered for tests and was thus much preferred on tests than the more subjective interpretations of how some data plot could be broken down to some equation or sets of equations—the latter for which there was typically no right answer (but also no wrong answer).

The one book you will want to acquire and hold onto (if you can find one) is titled "Enzyme Nomenclature" and it contains the "Recommendations (1972) of the International Union of Pure and Applied Chemistry (IUPAC) and the International Union of Biochemistry." My worn and beat-up book was copyrighted in 1973 by Elsevier Scientific Publishing Company, Amsterdam, and has been with me for over 40 years. There was a later version published by the IUPAC in 1981: "Symbolism and terminology in chemical kinetics" in *Pure and Applied Chemistry*, volume 53, 1981, pp. 753–771, but I think more recent information is available to you. In the "modern" computer-friendly age, you will find multiple resources for enzyme nomenclature and although I do not propose to represent the utility of a given resource, nor do I recommend one resource over another resource, I would refer you to several web sites such as:

Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB): Enzyme Nomenclature (www.chem.qmul.ac.uk/iubmb/enzyme/)
International Union of Biochemistry and Molecular Biology (www.iubmb.org/index.php?id=33)
Pure and Applied Chemistry: IUPAC Technical Reports and Recommendations (www.iupac.org/publications/pac/40/3/)
Science Direct (www.sciencedirect.com)
eLS citable reviews (www.els.net)

Some of these sites will require you to log on through a society membership or via other institutional login options. In some cases you are also able to purchase online access for a given period of time. The International Union of Biochemistry and Molecular Biology (IUBMB), formerly the International Union of Biochemistry or IUB, suggests that although both kinetic recommendations and enzyme nomenclature are "surprisingly" attributed to the International Union of Pure and Applied Chemistry (IUPAC) that both are in reality the exclusive responsibility of the IUBMB (but, expert chemists are, of course, consulted when appropriate).

You can, of course, get bogged down in any one of several good published narratives describing the pros and cons of how to express rate constants, concentrations of "substrates" (reactants), and concentrations of "products" in some enzymatic reaction, but for a book on the basics of enzymology, I have chosen to keep such expressions as simple as possible (it also will help you in inputting

such expressions on the printed page without superscripts, subscripts, etc.—at least as much as possible). You will note, for example, that I have avoided and will continue to avoid the use of the rate constant typically called K_{cat} (or k_{kat}), which is commonly used in textbooks and rate equations. I have chosen to keep you to quasi-equilibrium assumptions and use a rate constant such as "k_2" (or similar expressions) as the rate-limiting step in the conversion of substrate to product; in defining it as the rate-limiting step it is easy for you to understand that this rate constant (or similar such expressions for a rate-limiting rate constant) define the catalytic rate (or turnover number) by which an enzyme converts substrate to product. You should appreciate, however, that when it comes time for you to publish in some journal, it will be your responsibility to read the instructions to authors and adhere to the requirements of that journal for how you describe and define any symbolism you chose to use.

Anyway, let's move on to an overview of enzyme nomenclature that you will need so as to not embarrass yourself as you participate in classes in enzymology or biochemistry and ask questions of the professor. Enzymes are typically identified by either their trivial or recommended names. The trivial names of enzymes are favored by most researchers as being more descriptive and more easily written in publications. Enzymes are typically assigned a code number (which are widely used) and such code numbers contain four elements separated by points within the following meanings:

i) The first number shows to which of the six main divisions (classes) the enzyme belongs.
ii) The second number indicates the sub-class to which the enzyme belongs.
iii) The third number gives the sub-sub-class to which the enzyme belongs.
iv) The fourth number is the serial number of the enzyme in its sub-sub-class.

I appreciate that this code system sounds awkward, but I think you will find it illuminating as we progress into the classification and coding scheme. The main divisions and sub-classes of enzymes are as follow.

1) **Oxidoreductases:** To this division belong all enzymes catalyzing oxidation-reduction reactions. The sub-class of oxidoreductases (the second number) indicates the group in the hydrogen donor that undergoes oxidation. The sub-sub-class number (the third number) indicates the type of acceptor involved.
2) **Transferases:** To this division belong all enzymes transferring a group from one compound to another compound. The sub-class of transferases (the second number) indicates the group transferred, and the sub-sub-class provides additional information on the group transferred.
3) **Hydrolases:** To this division belong those enzymes that catalyze the hydrolytic cleavage of C–O, C–N, C–C, and some other bonds including phosphoric anhydride bonds. The sub-class (second number) of hydrolases indicates the nature of the bond hydrolyzed, and the sub-sub-class (the third number) specifies the nature of the substrate.
4) **Lyases:** To this division belong those enzymes that cleave C–C, C–O, C–N, and other bonds by elimination, leaving double bonds (or conversely adding groups to double bonds). The sub-class (second number) indicates the bond

broken, and the sub-sub-class (third number) gives further information on the group eliminated.

5) **Isomerases:** To this division belong those enzymes that catalyze geometric or structural changes within one molecule (these include such generic names as racemases, epimerases, isomerases, mutases, etc.). The sub-class (second number) corresponds to the type of isomerism performed, and the sub-sub-class (third number) is based on the substrate(s) involved in the isomerization.
6) **Ligases (synthetases):** To this division belong those enzymes that catalyze the joining together of two molecules coupled with the hydrolysis of a pyrophosphate bond in ATP or a similar triphosphate. The sub-class (second number) indicates the bond formed, and the sub-sub-class (third number) is only used in the C–N ligases.

Just as an example, I will try to illustrate this numbering code for transferases by giving you a few numbering schemes. For example, transferases begin with the first number 2, and a transferase that transfers one-carbon groups would carry the second number 1. Thus you could have four sub-sub-classes within this group of transferases that transfer one-carbon groups:

2.1.1 methyltransferases
2.1.2 hydroxymethyl, formyl, and related transferases
2.1.3 carboxyl- and carbamoyltransferases
2.1.4 amidinotransferases

Finally, a transferase with the code of 2.1.1.1 is by definition a methyltransferase, and in this instance is a nicotinamide methyltransferase where *S*-adenosyl-L-methionine plus nicotinamide becomes *S*-adenosyl-L-homocysteine plus 1-methylnicotinamide.

I could go on here describing how enzyme nomenclature evolved, but I would only be reciting the words of individuals much more qualified in enzymology than I am and you would stop reading the book at this point. My purpose in giving you this information here is to help you understand some of the definitions and expressions you will encounter in the study of enzymology (and biochemistry).

Activity Coefficients

As a final attempt at "definitions" that are relevant to the study of enzymes, I would like to introduce you to the concept of an "activity coefficient" (which I alluded to earlier). I first encountered "activity coefficient" as a graduate student when I made my buffers according to the Henderson–Hasselbalch equation, but had to continually adjust the final pH of the buffer. I soon learned the difference between concentrations I measured by weight and volume and "real concentrations" I needed to achieve the desired pH. If you read Wikipedia (Wikipedia.org) you will find a definition for activity coefficient that reads, "An activity coefficient is a factor used in thermodynamics to account for

deviations from ideal behavior in a mixture of chemical substances." Basically, the premise here is that in an *ideal* situation, interactions between two chemical species (an enzyme and substrate as an example) are the same (meaning, e.g., that size does not matter) and thus properties of a chemical interaction can be expressed directly in terms of simple concentrations. However, few chemical mixtures (solutions) behave in an "ideal" manner (e.g., they behave the same no matter what their concentrations, what solution(s) they are in, or what other chemicals are present in that solution) and thus deviations from ideality need to be accommodated by modifying the concentration by use of an activity coefficient. The concept of activity coefficient is closely linked to that of activity in chemistry, where activity relates to the propensity of a chemical to engage in some chemical reaction (its "effective concentration") and is derived as the product of concentration and an activity coefficient of the chemical engaging in some chemical interaction—which is, of course, what a substrate engaging with an enzyme is all about.

For purposes of this book, I will treat the activity coefficient as approaching unity. This means the value (which is dimensionless) of an activity coefficient for all reactants being discussed will be equal to 1, and therefore any concentrations discussed will represent the effective concentration of that reactant. This is not the time to get into such details regarding how the concentration of a substrate may not be the effective concentration of that substrate; however, you will need to be aware of the differences between a concentration and an effective concentration when it comes to dealing with enzyme kinetics and rate constants in your research. If you wish to obtain specific activity coefficients I would refer you to http://sites.google.com/…/activity-coefficient/. However, you will be wise to understand that activity coefficients of a chemical (enzymatic) reactant can change relative to the concentrations of the reactants in the chemical and/or enzymatic event, and reactants at very dilute concentrations may have activity coefficients approaching unity, but very different activity coefficients at high concentrations. This is why enzymology (working with enzyme kinetics and reaction rates) is difficult.