Chapter 4

# Catalysis

**In living systems, speed is everything. Providing the reaction speeds necessary to support life are the catalysts, mostly in the form of enzymes.**

# Catalysis



## Introduction

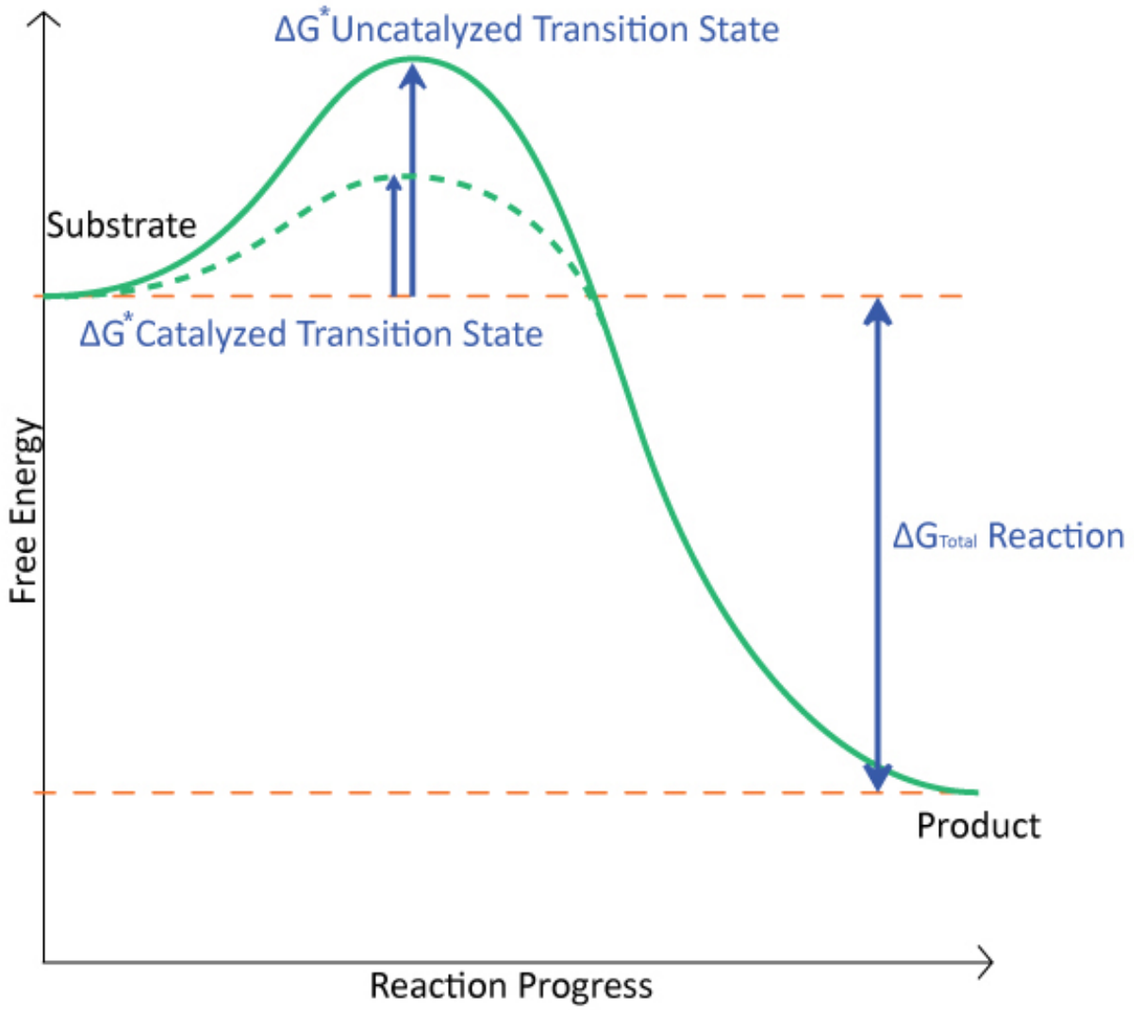
If there is a magical component to life, an argument can surely be made for it being **catalysis**. Thanks to catalysis, reactions that could take hundreds of years to complete in the "real world," occur in seconds in the presence of a catalyst. Chemical catalysts, like platinum, speed reactions, but **enzymes** (which are simply super-catalysts with a twist) put chemical catalysts to shame. To understand enzymatic catalysis, we must first understand energy. In Chapter 2, we noted the tendency for processes to move in the direction of lower energy. Chemical reactions follow this universal trend, but they often have a barrier in place that must be overcome. The secret to catalytic action is reducing the magnitude of that barrier, as we shall see.

| Enzyme | Nonenzymatic Half-Life | Uncatalyzed Rate ($k_{un}$ $s^{-1}$) | Catalyzed Rate ($k_{cat}$ $s^{-1}$) | Rate Enhancement ($k_{cat}$ $s^{-1}/k_{un}$ $s^{-1}$) |
|---|---|---|---|---|
| OMP decarboxylase | 78,000,000 years | $28 \times 10^{-16}$ | 39 | $1.4 \times 10^{17}$ |
| Staphylococcal nuclease | 130,000 years | $1.7 \times 10^{-13}$ | 95 | $5.6 \times 10^{14}$ |
| Carboxypeptidase A | 7.3 years | $3.0 \times 10^{-9}$ | 578 | $1.9 \times 10^{11}$ |
| Ketosteroid isomerase | 7 weeks | $1.7 \times 10^{-7}$ | 66,000 | $3.9 \times 10^{11}$ |
| Triose phosphate isomerase | 1.9 days | $4.3 \times 10^{-6}$ | 4,300 | $1.0 \times 10^{9}$ |
| Chorismate mutase | 7.4 hours | $2.6 \times 10^{-5}$ | 50 | $1.9 \times 10^{6}$ |
| Carbonic anhydrase | 5 seconds | $1.3 \times 10^{-1}$ | $1 \times 10^{6}$ | $7.7 \times 10^{6}$ |

Enzyme Rate Enhancements

# Activation Energy

The figure below schematically depicts the energy changes that occur during the progression of a simple reaction. In the figure, the energy differences *during* the reaction are compared for a catalyzed (plot on the right) and an uncatalyzed reaction (plot on the left). Notice that the reactants start at the same energy level for both conditions and that the products end at the same energy for both as well. Thus, the difference in energy between the energy of the ending compounds and the starting compounds is the same in both cases. This is the first important rule to understand any kind of catalysis – catalysts do not change the overall energy of a reaction. Given enough time, a non-catalyzed reaction will get to the same equilibrium as a catalyzed one.




Energetic Considerations of Catalysis

Another feature to note about catalyzed reactions is the reduced energy barrier (also called the **activation energy** or free energy of activation) to reach the transition state of the catalyzed reaction.

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This is the second important point about catalyzed reactions – catalysts work by lowering activation energies of reactions and thus molecules more easily reach the energy necessary to get to the point where the reaction occurs. Note that these reactions are reversible. The extent to which they will proceed is a function of the size of the energy difference between the product and reactant states. The lower the energy of the products compared to the reactants, the larger the percentage of molecules that will be present as products at equilibrium. At equilibrium, of course, no change in concentration of reactants and products occurs because at this point, the forward and reverse reaction rates are the same.

## General Mechanisms of Action

As noted above, enzymes are orders of magnitude more effective (faster) than chemical catalysts. The secret of their success lies in a fundamental difference in their mechanisms of action. Every chemistry student has had hammered into their heads the fact that a catalyst speeds a reaction without being consumed by it. In other words, the catalyst ends up after a reaction just the way it started so it can catalyze other reactions, as well. Enzymes share this property, but in the middle, during the catalytic action, an enzyme is transiently changed. Such changes may be subtle electronic ones or more significant covalent modifications. It is also important to recognize that enzymes are not fixed, rigid structures, but rather are flexible. Flexibility allows movement and movement facilitates alteration of electronic environments necessary for catalysis. Enzymes are, thus, much more efficient than rigid chemical catalysts as a result of their abilities to facilitate the changes necessary to optimize the catalytic process.

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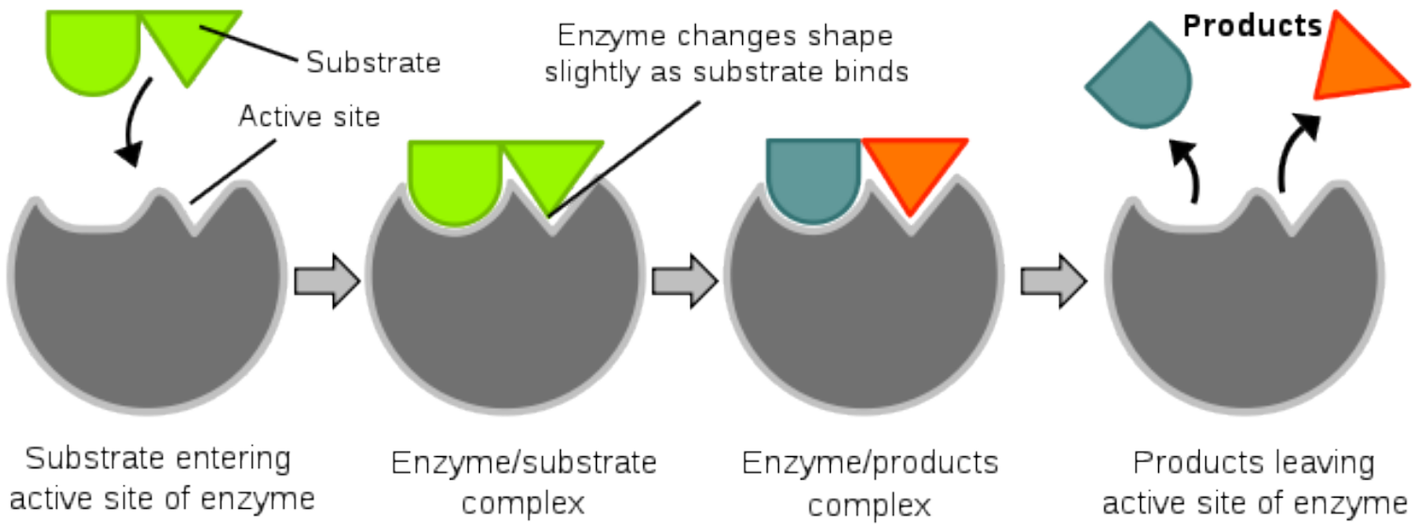
## Substrate Binding

Another important difference between the mechanism of action of an enzyme and a chemical catalyst is that an enzyme has binding sites that not only 'grab' the **substrate** (molecule involved in the reaction being catalyzed), but also place it in a position to be electronically induced to react, either within itself or with another substrate. The enzyme itself may play a role in the electronic induction or the induction may occur as a result of substrates being placed in very close proximity to each other. Chemical catalysts have no such ability to bind substrates and are dependent upon them colliding in the right orientation at or near their surfaces.




Mechanism of Induced Fit

## The Way They Work

To the tune of "*The Way We Were*"

Enzymes
Mighty powerhouse peptides
Cause reactions to go faster
In the cell's insides

Tiny substrates
Bring about an induced fit
Enzyme structure is affect-ed
By what binds to it

Can it be that it's just simple zen?
How the enzymes activate
If they bind effector, will they go
To an R-State, T-State?

Folding
Gives the mechanistic might
To three-D arrangement
Of the active site

Enzymes
Have a bias they can't hide
Hydrophobic side chains are
Mostly found inside

So it's the structure
For celebrating
Whenever there's debating
The way they work

*Recorded by Liz Bacon and David Simmons*
*Lyrics by Kevin Ahern*

## Enzyme Flexibility

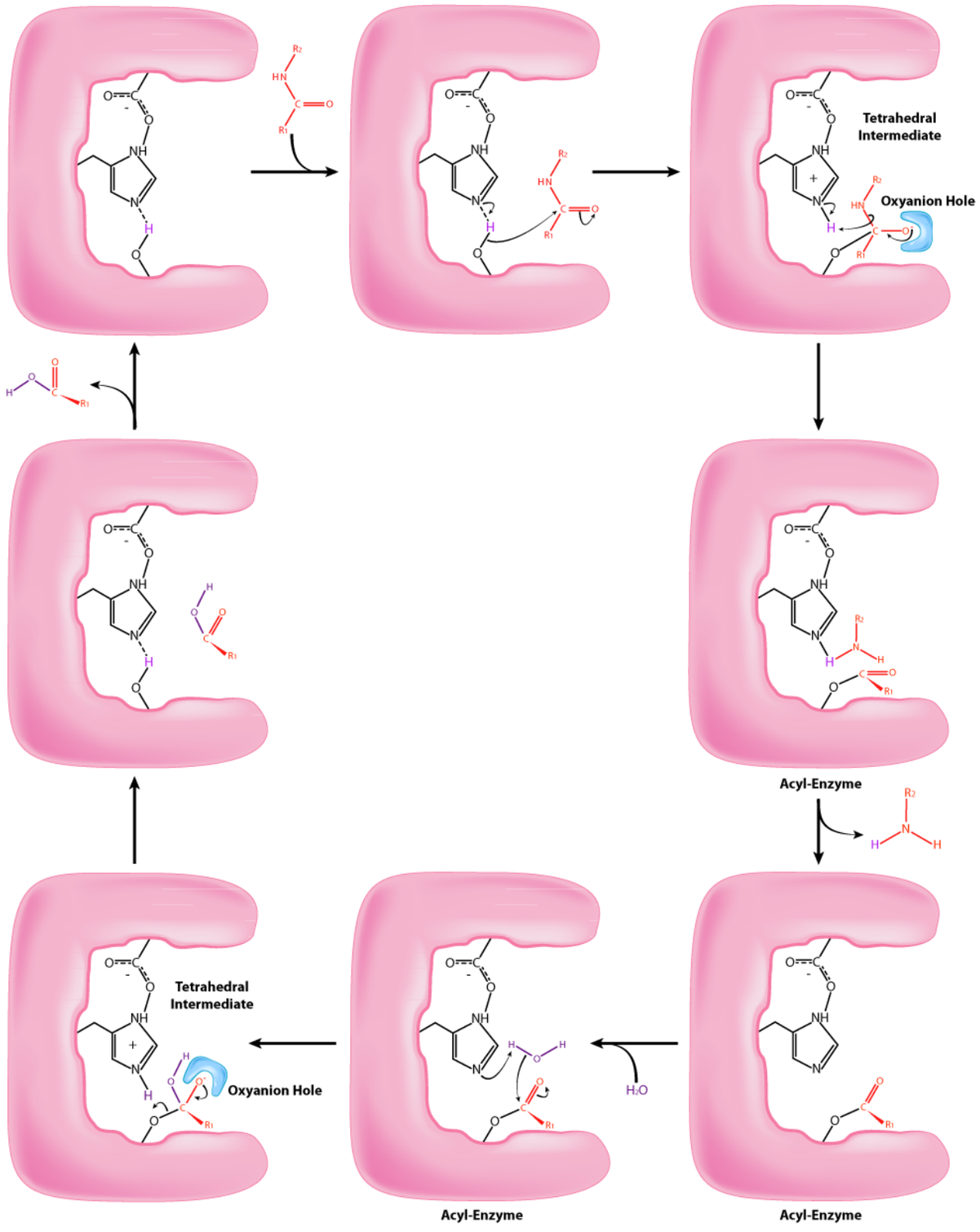
As mentioned earlier, a difference between an enzyme and a chemical catalyst is that an enzyme is flexible. Its slight changes in shape (often arising from the binding of the substrate itself) help to position substrates for reaction after they bind. These changes in shape are explained, in part, by **Koshland's Induced Fit Model of Catalysis**, which illustrates that not only do enzymes change substrates, but that substrates also transiently change enzymes. At the end of the catalysis, the enzyme is returned to its original state. Enzyme flexibility also is important for control of enzyme activity. Two distinct structures are typically described– the T (tight) state, which is a lower activity state and the R (relaxed) state, which has greater activity.

## Active Site

Reactions in enzymes are catalyzed at a specific location known as the '**active site**'. Substrate binding sites are located in close physical proximity to the active site and oriented to provide access for the relevant portion of the molecule to the electronic environment of the enzyme where catalysis is initiated.

# Chymotrypsin

Consider the mechanism of catalysis of the enzyme known as **chymotrypsin**. Found in our digestive system, chymotrypsin's catalytic action is cleaving peptide bonds in proteins and it uses the side chain of a serine in its mechanism of catalysis. Many other protein-cutting enzymes employ a very similar mechanism and they are known collectively as **serine proteases**. As a **protease**, it acts fairly specifically, cutting not all peptide bonds, but only those that are adjacent to specific amino acids in the protein. One of the amino acids it cuts adjacent to is phenylalanine. The enzyme's action occurs in two phases – a fast phase that occurs first and a slower phase that follows. The enzyme has a substrate binding site that includes a region of the enzyme known as the **S1 pocket**. Let us step through the mechanism by which chymotrypsin cuts adjacent to phenylalanine.




Serine Protease Mechanism

The process starts with the binding of the substrate in the S1 pocket. The S1 pocket in chymotrypsin has a hydrophobic hole in which the substrate is bound. Preferred substrates will include amino acid side chains that are hydrophobic, like phenylalanine. If an ionized side chain, like that of glutamic acid binds in the S1 pocket, it will quickly exit, much like water would avoid an oily interior. When the proper substrate binds, it stays and its presence induces an ever so slight shift in the shape of the enzyme. This

## The Serine Protease Song

To the tune of "*Blackbird*"

Substrate floating in the cell's insides
Enzyme snags it with its binding site
It supplies
Shuffling of electrons in the act to catalyze

Proteases of the serine kind
Break up peptide bonds in rapid time
Fast and slow
Steps in breaking bonds are mechanisms you should know

Asp – his - ser
Bonds beware
Inside the S1 pocket substrate sits

Alkoxides
Break peptides
Nucleophiles give bonds the fits

Peptide one exits easily
But water has to let the other flee
Bound not free
'Cuz the enzyme's linked to it in mechanism three

When it's gone the enzyme's free to catalyze you see
When it's gone the enzyme's free to catalyze you see

*Recorded by David Simmons*
*Lyrics by Kevin Ahern*

subtle shape change on the binding of the proper substrate starts the steps of the catalysis and is the reason that the enzyme shows specificity for cutting at specific enzyme positions in the target protein. Only amino acids with the side chains that interact well with the S1 pocket start the catalytic wheels turning.

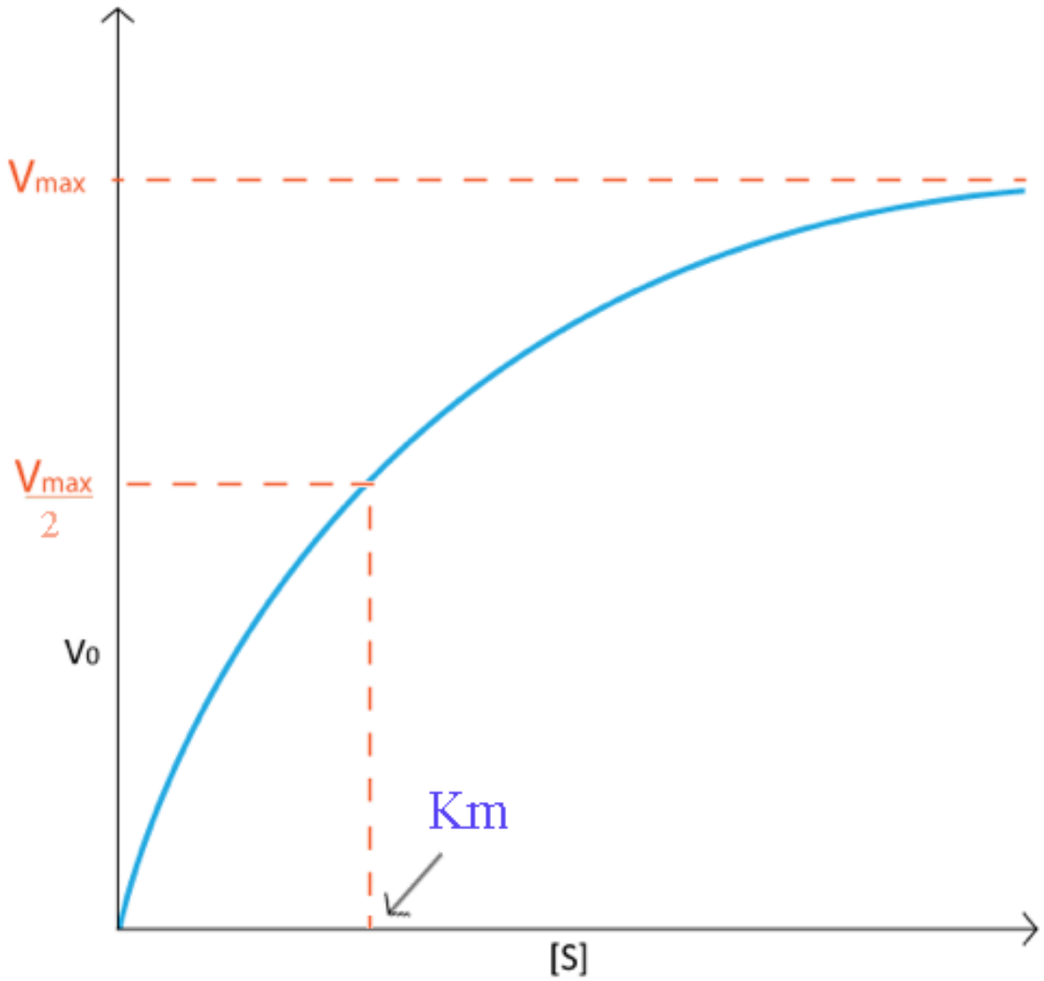
The slight changes in shape of the enzyme upon binding of the proper substrate cause changes in the positioning of three amino acids (aspartic acid, histidine, and serine) in the active site known as the **catalytic triad**, during the second step of the catalytic action. The shift of the negatively charged aspartic acid towards the electron rich histidine ring favors the abstraction of a proton by the histidine from the hydroxyl group on the side chain of serine, resulting in production of a very reactive **alkoxide ion** in the active site. Since the active site at this point also contains the polypeptide chain positioned with the phenylalanine side chain embedded in the S1 pocket, the alkoxide ion performs a **nucleophilic attack** on the peptide bond on the carboxyl side of phenylalanine sitting in the active site. This reaction, which is the third step of catalysis, breaks the bond and causes two things to happen. First, one end of the original polypeptide is freed and exits the active site. The second is that the end containing the phenylalanine is covalently linked to the oxygen of the serine side chain. At this point we have completed the first (fast) phase of the catalysis.

The second phase of the catalysis by chymotrypsin is slower. It requires that the covalent bond between phenylalanine and serine's oxygen be broken so the peptide can be released and the enzyme can return to its original state. The process starts with entry of water into the active site. Water is attacked in a fashion similar to that of the serine side chain in the first phase, creating a reactive hydroxyl group that performs a nucleophilic attack on the phenylalanine-serine bond, releasing it and replacing the proton on serine. The second peptide is released in the process and the reaction is complete with the enzyme back in its original state.

## Enzyme Parameters

Scientists spend a considerable amount of time characterizing enzymes. To understand how they do this and what the characterizations tell us, we must first understand a few parameters. Imagine I wished to study the reaction catalyzed by an enzyme I have just isolated. I would be interested to understand how fast the enzyme works and how much affinity the enzyme has for its substrate(s).

To perform this analysis, I would perform the following experiment. Into 20 different tubes, I would put enzyme buffer (to keep the enzyme stable), the same amount of enzyme, and then a different amount of substrate in each tube, ranging from tiny amounts in the first tubes to very large amounts in the last tubes. I would let the reaction proceed for a fixed, short amount of time and then I would measure the amount of product contained in each tube. For each reaction, I would determine the velocity of the reaction as the concentration of product found in each tube divided by the time. I would then plot the data on a graph using velocity on the Y-axis and the concentration of substrate on the X-axis.




Velocity vs Substrate Concentration Plot

Typically, I would generate a

curve like that shown at the left. Notice how the velocity increase is almost linear in the tubes with the lowest amounts of substrate. This indicates that substrate is limiting and the enzyme converts it into product as soon as it can bind it. As the substrate concentration increases, however, the velocity of the reaction in tubes with higher substrate concentration ceases to increase linearly and instead begins to flatten out, indicating that as the substrate concentration gets higher and higher, the enzyme has a harder time keeping up to convert the substrate to product. What is happening is the enzyme is becoming saturated with substrate at higher concentrations of the latter. Not surprisingly, when the enzyme becomes completely saturated with substrate, it will not have to wait for substrate to diffuse to it and will therefore be operating at maximum velocity.

| Enzyme | Turnover Number (per second) |
|---|---|
| Carbonic anhydrase | 600,000 |
| 3-Ketoesteroid isomerase | 280,000 |
| Acetylcholinesterase | 25,000 |
| Penicillinase | 2,000 |
| Lactate dehydrogenase | 1,000 |
| Chymostrypsin | 100 |
| DNA Polymerase I | 15 |
| Tryptophan synthetase | 2 |
| Lysozyme | 0.5 |

## $V_{max}$ and $K_{cat}$

On a plot of Velocity versus Substrate Concentration (**V vs. [S]**), the maximum velocity (known as $\mathbf{V_{max}}$) is the value on the Y axis that the curve asymptotically approaches. It should be noted that the value of $V_{max}$ depends on the amount of enzyme used in a reaction. Double the amount of enzyme, double the $V_{max}$. If one wanted to compare the velocities of two different enzymes, it would be necessary to use the same amounts of enzyme in the different reactions they catalyze. It is desirable to have a measure of velocity that is independent of enzyme concentration. For this, we define the value $\mathbf{K_{cat}}$, also known as the turnover number. Mathematically,

$$K_{cat} = V_{max} / [Enzyme]$$

To determine $K_{cat}$, one must obviously know the $V_{max}$ at a particular concentration of enzyme, but the beauty of the term is that it is a measure of velocity independent of enzyme concentration, thanks to the term in the denominator. $K_{cat}$ is thus a constant for an enzyme under given conditions. The

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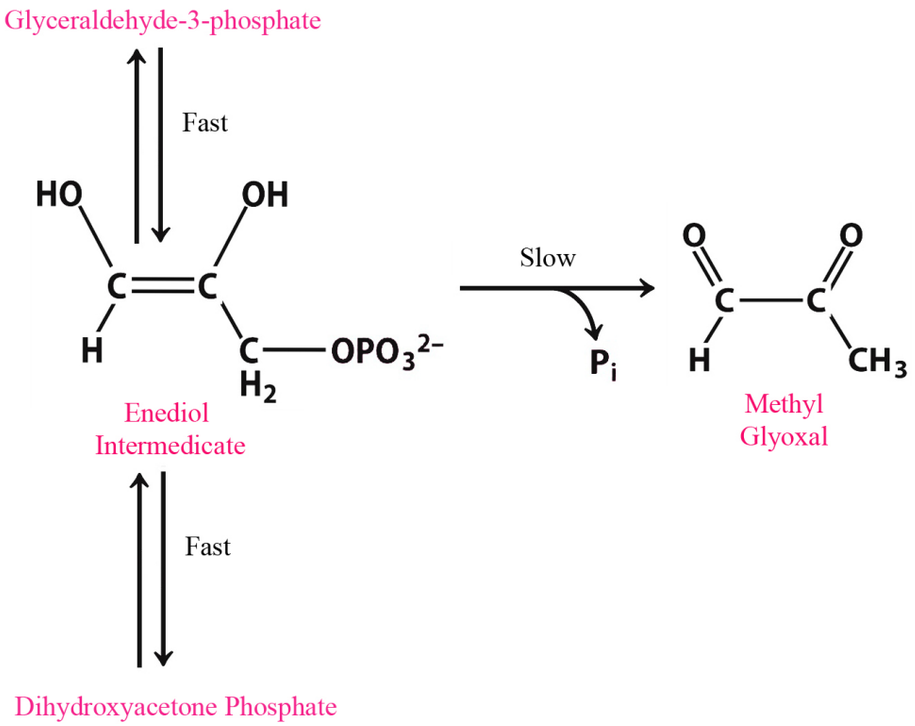
units of $K_{cat}$ are time$^{-1}$. An example would be 35/second. This would mean that each molecule of enzyme is catalyzing the formation of 35 molecules of product every second. While that might seem like a high value, there are enzymes known (**carbonic anhydrase**, for example) that have Kcat values of $10^6$/second. This astonishing number illustrates clearly why enzymes seem almost magical in their action.

## $K_M$

Another parameter of an enzyme that is useful is known as **$K_M$**, the Michaelis constant. What it measures, in simple terms, is the affinity an enzyme has for its substrate. Affinities of enzymes for substrates vary considerably, so knowing $K_M$ helps us to understand how well an enzyme is suited to the substrate being used. Measurement of $K_M$ depends on the measurement of $V_{max}$. On a V vs. [S] plot, $K_M$ is determined as the x value that gives Vmax /2.

A common mistake students make in describing $V_{max}$ is saying that $K_M$ = $V_{max}$ /2. This is, of course not true.




**Avoidance of Formation of an Unstable Intermediate in Triose Phosphate Isomerase**

$K_M$ is a substrate concentration and is the amount of substrate it takes for an enyzme to reach $V_{max}$ /2. On the other hand $V_{max}$ /2 is a velocity and is nothing more than that. The value of $K_M$ is inversely related to the affinity of the enzyme for its substrate. High values of $K_M$ correspond to low enzyme affinity for substrate (it takes more substrate to get to $V_{max}$). Low $K_M$ values for an enzyme correspond to high affinity for substrate.

## Perfect Enzymes

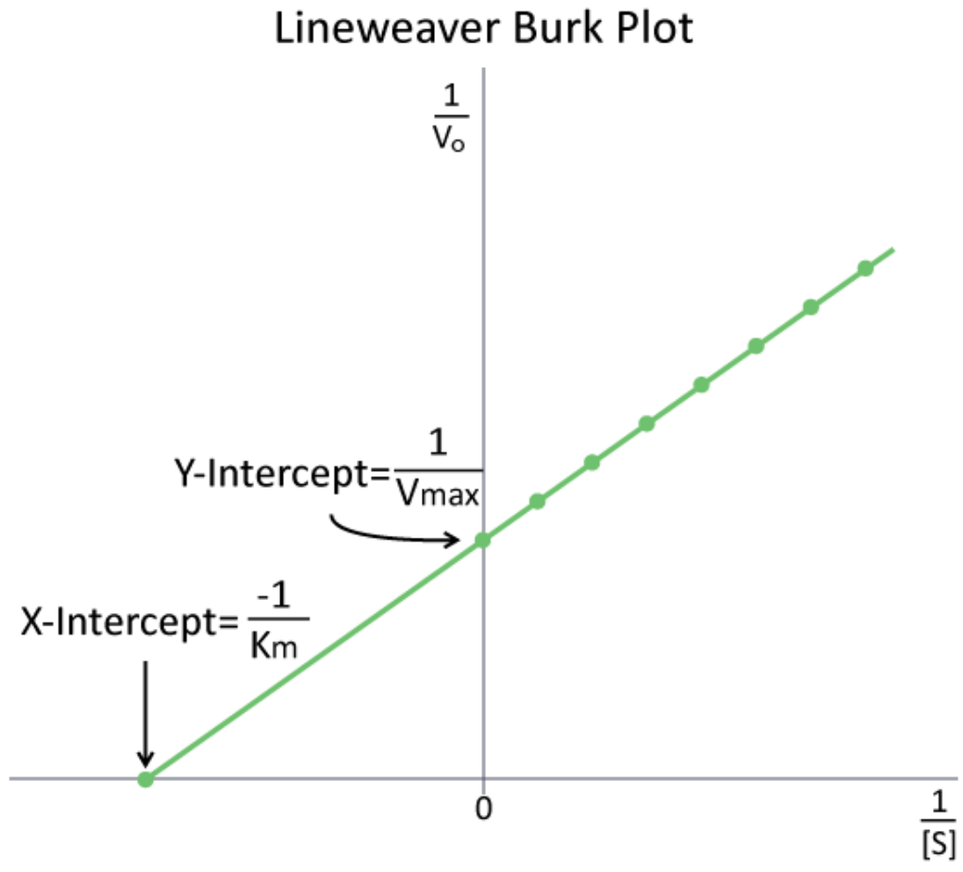
Now, if we think about what an ideal enzyme might be, it would be one that has a very high velocity and a very high affinity for its substrate. That is, it wouldn't take much substrate to get to $V_{max}$/2 and the $K_{cat}$ would be very high. Such enzymes would have values of $K_{cat}$ / $K_M$ that are maximum. Interestingly, there are several enzymes that have this property and their maximal values are all approximately the same. Such enzymes are referred to as being "perfect" because they have reached the maximum possible value. Why should there be a maximum possible value of $K_{cat}$ /

$K_M$? The answer is that movement of substrate to the enzyme becomes the limiting factor for perfect enzymes. Movement of substrate by diffusion in water has a fixed rate and that limitation ultimately determines how fast the enzyme can work. In a macroscopic world analogy, factories can't make products faster than suppliers can deliver materials. It is safe to say for a **perfect enzyme** that the only limit it has is the rate of substrate diffusion in water.

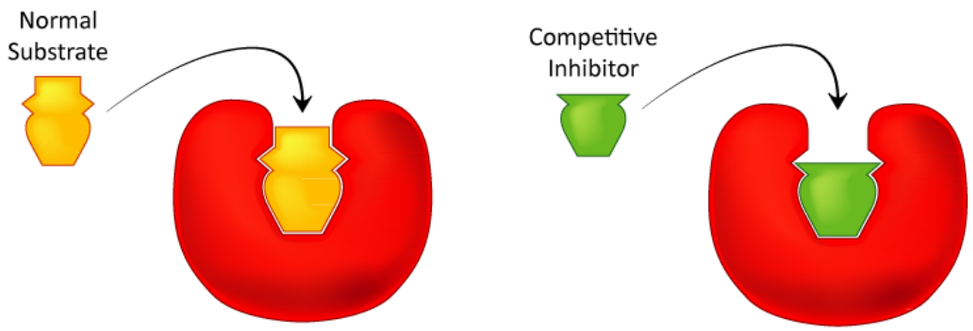
Given the "magic" of enzymes alluded to earlier, it might seem that all enzymes should have evolved to be "perfect." There are very good reasons why most of them have not. Speed can be a dangerous thing. The faster a reaction proceeds in catalysis by an enzyme, the harder it is to control. As we all know from learning to drive, speeding causes accident. Just as drivers need to have speed limits for operating automobiles, so too must cells exert some control on the 'throttle' of their enzymes. In view of this, one might wonder then why any cells have evolved any enzymes to perfection. There is no single answer to the question, but a common one is illustrated by the perfect enzyme known as triose phosphate isomerase (TPI), which catalyzes a reaction in glycolysis (figure on previous page). The enzyme appears to have been selected for this ability because at lower velocities, there is breakdown of an unstable enediol intermediate that then readily forms methyl glyoxal, a cytotoxic compound. Speeding up the reaction provides less opportunity for the unstable intermediate to accumulate and fewer undesirable byproducts are made.

## Lineweaver-Burk Plots

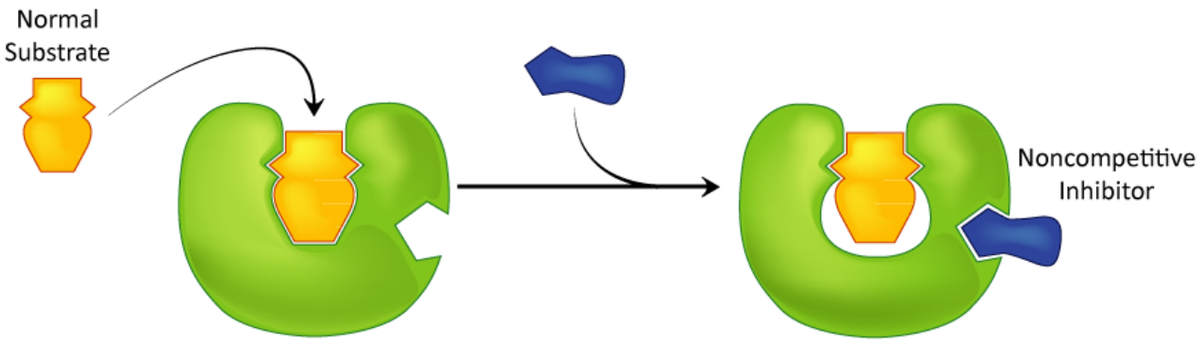
The study of enzyme kinetics is typically the most math intensive component of biochemistry and one of the most daunting aspects of the subject for many students. Although attempts are made to simplify the mathematical considerations, sometimes they only serve to confuse or frustrate students. Such is the case with modified enzyme plots, such as **Lineweaver-Burk** (left). Indeed, when presented by professors as simply another thing to




Lineweaver-Burk Plot



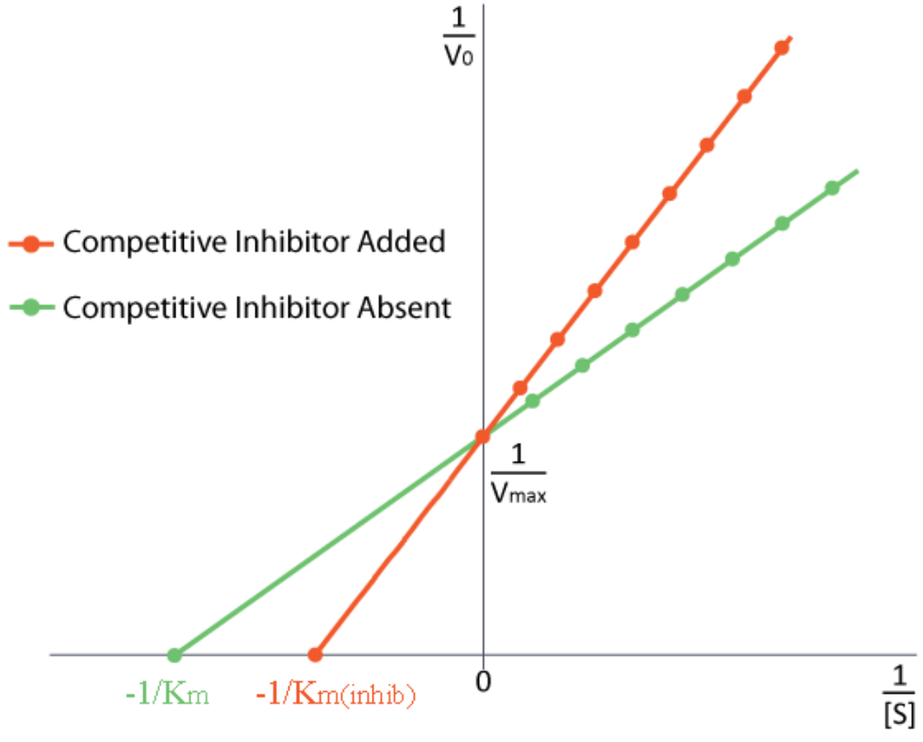

Competitive Inhibition




Non-Competitive Inhibition

memorize, who can blame students? In reality, both of these plots are aimed at simplifying the determination of parameters, such as $K_M$ and $V_{max}$. In making either of these modified plots, it is important to recognize that the same data is used as in making a V vs. [S] plot. The data are simply manipulated to make the plotting easier.

For a LineWeaver-Burk, the manipulation is using the reciprocal of the values of both the velocity and the substrate concentration. The inverted values are then plotted on a graph as **1/V vs. 1/[S]**. Because of these inversions, Lineweaver-Burk plots are commonly referred to as 'double-reciprocal' plots. As can be seen at left, the value of $K_M$ on a Lineweaver Burk plot is easily determined as the negative reciprocal of the x-intercept , whereas the Vmax is the inverse of the y-intercept. Other related manipulation of kinetic data include Eadie-Hofstee diagrams, which plot V vs V/[S] and give Vmax as the Y-axis intercept with the slope of the line being - $K_M$.




Lineweaver-Burk Plot of Competitive Inhibition

## Enzyme Inhibition

Inhibition of specific enzymes by drugs can be medically useful. Understanding the mechanisms of enzyme inhibition is therefore of considerable importance. We will discuss four types of enzyme inhibition – competitive, non-competitive, uncompetitive, and suicide. Of these, the first three types are reversible. The last one is not.

# Competitive Inhibition

Probably the easiest type of enzyme inhibition to understand is **competitive inhibition** and it is the one most commonly exploited pharmaceutically. Molecules that are competitive inhibitors of enzymes resemble one of the normal substrates of an enzyme. An example is methotrexate, which resembles the **folate** substrate of the enzyme **dihydrofolate reductase** (**DHFR**). This enzyme normally catalyzes the reduction of folate, an important reaction in the metabolism of nucleotides. When the drug **methotrexate** is present, some of the enzyme binds to it instead of to folate and during the time methotrexate is bound, the enzyme is inactive and unable to bind folate. Thus, the enzyme is inhibited. Notably, the binding site on DHFR for methotrexate is the active site, the same place that folate would normally bind. As a result, methotrexate 'competes' with folate for binding to the enzyme. The more methotrexate there is, the more effectively it competes with folate for the enzyme's active site. Conversely, the more folate there is, the less of an effect methotrexate has on the enzyme because folate outcompetes it.

## Enzymes

To the tune of "*Downtown*"

Reactions alone
Could starve your cells to the bone
Thank God we all produce
Enzymes

Units arrange
To make the chemicals change
Because you always use
Enzymes

Sometimes mechanisms run like they are at the races
Witness the Kcat of the carbonic anhydrases

How do they work?
Inside of the active site
It just grabs onto a substrate
and squeezes it tight
In an
ENZYME!
CAT-al-y-sis
In an
ENZYME!
V versus S
In an
ENZYME!
All of this working for you
(Enzyme, enzyme)

Energy peaks
Are what an enzyme defeats
In its catalysis
Enzymes

Transition state
Is what an enzyme does great
And you should all know this

Enzymes

Catalytic action won't run wild - don't get hysteric
Cells can throttle pathways with an enzyme allosteric

You know it's true
So when an effector fits
It will just rearrange
all the sub-u-nits
Inside an
ENZYME!
Flipping from R to T
ENZYME!
Slow catalytically
ENZYME!
No change in Delta G
(Enzyme, enzyme)

You should relax
When seeking out the Vmax though
There are many steps
Enzymes

Lineweaver Burk
Can save a scientist work
With just two intercepts
Enzymes

Plotting all the data from kinetic exploration
Lets you match a line into a best fitting equation

Here's what you do
Both axes are inverted then
You can determine Vmax and
Establish Km
for your
ENZYMES!
Sterically holding tight
ENZYMES!
Substrates positioned right
ENZYMES!
Inside the active site
Enzymes (Enzymes, enzymes, enzymes)

*Recorded by Barbara and Neal Gladstone*
*Lyrics by Kevin Ahern*

## No Effect on $V_{max}$

How do we study competitive inhibition? It is typically done as follows. First one performs a set of V vs. [S] reactions without inhibitor (20 or so tubes, with buffer and constant amounts of enzyme, varying amounts of substrate, equal reaction times). V vs. [S] is plotted, as well as 1/V vs. 1/[S], if desired. Next, a second set of reactions is performed in the same manner as before, except that a fixed amount of the methotrexate inhibitor is added to each tube. At low concentrations of substrate, the inhibitor competes for the enzyme effectively, but at high concentrations of substrate, the inhibitor will have a much reduced effect, since the substrate outcompetes it, due to its higher concentration (remember that the inhibitor is at fixed concentration). Graphically, the results of these experiments are shown above. Notice that at high substrate concentrations, the competitive inhibitor has essentially no effect, causing the $V_{max}$ for the enzyme to remain unchanged. To reiterate, this is due to the fact that at high substrate concentrations, the inhibitor doesn't compete well. However, at lower substrate concentrations it does.

For inhibition, here are rules
To give to students in the schools
Non-competers muddy facts
And drop the value of $V_{max}$
Competers, everyone should know
Will make the $K_M$ values grow
Uncompetition makes them think
Since both $K_M$ and $V_{max}$ shrink
And suicide covalently
Stops enzymes irreversibly

## Increased $K_M$

Note that the apparent $K_M$ of the enzyme for the substrate increases (-1/$K_M$ gets closer to zero - red line above) when the inhibitor is present, thus illustrating the better competition of the inhibitor at lower substrate concentrations. It may not be obvious why we call the changed $K_M$ the apparent $K_M$ of the enzyme. The reason is that the inhibitor doesn't actually change the enzyme's affinity for the folate substrate. It only appears to do so. This is because of the way that competitive inhibition works. When the competitive inhibitor binds the enzyme, it is effectively 'taken out of action.' Inactive enzymes have NO affinity for substrate and no activity either. We can't measure $K_M$ for an inactive enzyme.

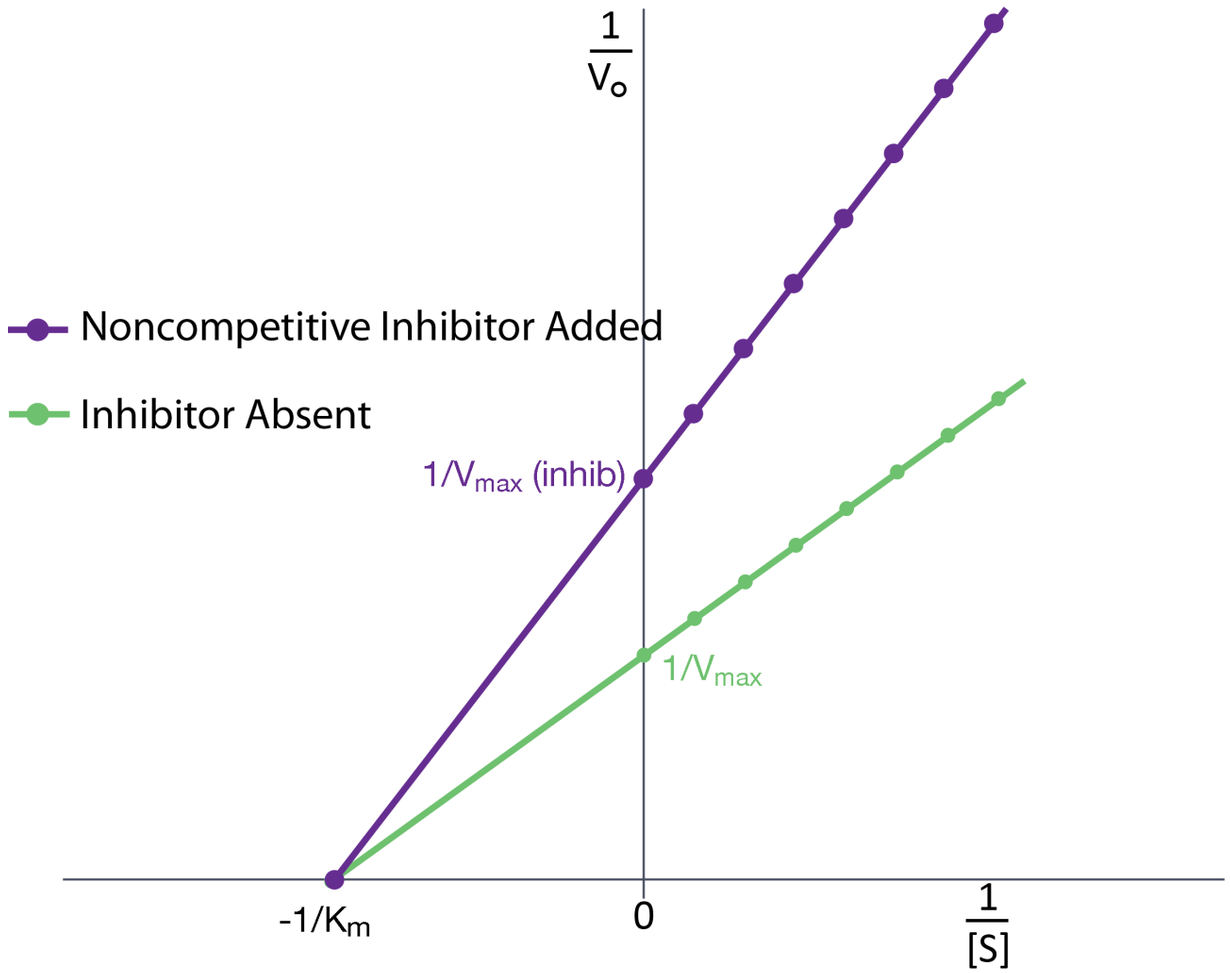
The enzyme molecules that are not bound by methotrexate can, in fact, bind folate and are active. Methotrexate has no effect on them and their $K_M$ values are unchanged. Why then, does $K_M$ appear higher in the presence of a competitive inhibitor? The reason is that the competitive inhibitor is reducing the amount of active enzyme at lower concentrations of substrate. When the amount of enzyme is reduced, one must have more substrate to supply the reduced amount of enzyme sufficiently to get to $V_{max}$ / 2.

It is worth noting that in competitive inhibition, the percentage of inactive enzyme changes drastically over the range of [S] values used. To start, at low [S] values, the greatest percentage of the enzyme is inhibited. At high [S], no significant percentage of enzyme is inhibited. This is not always the case, as we shall see in non-competitive inhibition.

## Non-Competitive Inhibition

A second type of inhibition employs inhibitors that do not resemble the substrate and bind not to the active site, but rather to a separate site on the enzyme (rectangular site below). The effect of binding a non-competitive inhibitor is significantly different from binding a competitive inhibitor because there is no competition. In the case of competitive inhibition, the effect of the inhibitor could be reduced and eventually overwhelmed with increasing amounts of substrate. This was because increasing substrate made increasing percentages of the enzyme active. With **non-competitive inhibition**, increasing the amount of substrate has no effect on the percentage of enzyme that is active. Indeed, in non-competitive inhibition, the percentage of enzyme inhibited remains the same through all ranges of [S].

This means, then, that non-competitive inhibition effectively reduces the amount of enzyme by the same fixed amount in a typical experiment at every substrate concentration used The effect of this inhibition is shown above. As you can see, $V_{max}$ is reduced in non-competitive inhibition compared to uninhibited reactions. This makes sense if we remember that $V_{max}$ is dependent on the amount of enzyme present. Reducing the amount of enzyme present reduces $V_{max}$. In competitive inhibition, this doesn't occur detectably, because at high substrate concentrations, there is essentially 100% of




Lineweaver-Burk Plot of Non-Competitive Inhibition

the enzyme active and the Vmax appears not to change. Additionally, $K_M$ for non-competitively inhibited reactions does not change from that of uninhibited reactions. This is because, as noted previously, one can only measure the $K_M$ of active enzymes and $K_M$ is a constant for a given enzyme.

## Uncompetitive Inhibition

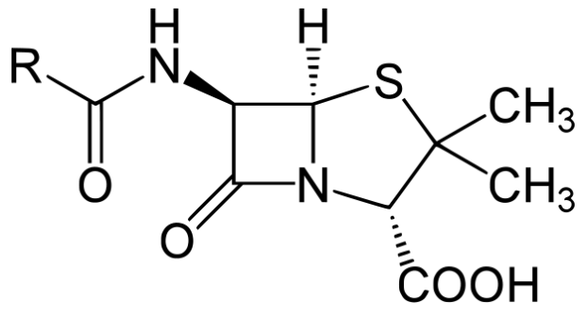
A third type of enzymatic inhibition is that of **uncompetitive inhibition**, which has the odd property of a reduced Vmax as well as a reduced $K_M$. The explanation for these seemingly odd results is rooted in the fact that the uncompetitive inhibitor binds only to the **enzyme-substrate (ES) complex**. The inhibitor-bound complex forms mostly under concentrations of high substrate and the ES-I complex cannot release product while the inhibitor is bound, thus explaining the reduced $V_{max}$.

The reduced $K_M$ is a bit harder to conceptualize. The answer lies in the fact that the inhibitor-bound complex effectively reduces the concentration of the ES complex. By Le Chatelier's Principle, a shift occurs to form additional ES complex, resulting in less free enzyme and more enzyme in the forms ES and ESI (ES with inhibitor). Decreases in free enzyme correspond to an enzyme with greater affinity for its substrate. Thus, paradoxically, uncompetitive inhibition both decreases Vmax and increases an enzyme's affinity for its substrate.

## Suicide Inhibition

In contrast to the first three types of inhibition, which involve reversible binding of the inhibitor to the enzyme, suicide inhibition is irreversible because the inhibitor becomes covalently bound to the enzyme during the inhibition and thus cannot be removed. Suicide inhibition rather closely resembles competitive inhibition because the inhibitor generally resembles the substrate and binds to the active site of the enzyme. The primary difference is that the suicide inhibitor is chemically reactive in the active site and makes a bond with it that precludes its removal. Such a mechanism is that employed by **penicillin** (right), which covalently links to the bacterial enzyme, **D-D transpeptidase** and stops it from functioning.

Since the normal function of the enzyme is to make a bond necessary for the **peptido-glycan complex** of the bacterial cell wall, the cell wall cannot properly form and bacteria cannot reproduce. If one were to measure the kinetics of suicide inhibitors under conditions where there was more enzyme than inhibitor, they would resemble non-competitive inhibition's kinetics because both involve reducing the amount of active enzyme by a fixed amount in a set of reactions.



Penicillin

# Control of Enzymes

It is appropriate that we talk at this point about mechanisms cells use to control enzymes. There are four general methods that are employed. They include 1) allosterism; 2) **covalent modification**; 3) access to substrate; and 4) control of enzyme synthesis/breakdown. Some enzymes are controlled by more than one of these methods.

## Allosterism

The term allosterism refers to the fact that the activity of certain enzymes can be affected by the binding of small molecules to the enzyme. In **allostery**, the molecules that are binding are non-substrate molecules that bind at a place on the enzyme other than the active site.

An excellent example of allosteric control is the regulation of **HMG-CoA reductase**, which catalyzes an important reaction in the pathway leading to the synthesis of cholesterol. Binding of **cholesterol** to the enzyme reduces the enzyme's activity significantly. Cholesterol

## Catalyze

To the tune of "*Close to You*"

My enzymes
Truly are inclined
To convert
Things they bind
Turn the key
Covalently
Cat-a-lyze

How do cells
Regulate these roles?
Allo-ster
-ic controls
Two forms, you see
States R and T
Mod-u-late

Competing inhibition keeps
The substrates from the active site
They raise Km, but leave Vmax and shirk
While the non-competers bind elsewhere
And lift the plot made on Lineweaver-Burk

Other ways
Enzymes can be blocked
When things bind
Then get locked
Stuck not free

Tied to the key
Su-i-cid

Penicillin's action stops
Peptidoglycan cross-links in
Bacterial cell walls in awesome ways
Beta lactam ring's reactive site
Starts bonding with D-D-transpeptidase

So there are
Several enzyme states
Counteract
-ing substrates
Now you see
Blocking the key
Regulates

Cat-a-lysts
Have to be controlled
Some get slowed
Put on hold
It's sublime
How the enzymes
(slow) Cat-a-lyze

It's sublime
How the enzymes
(slow) Cat-a-lyze

*Recorded by Barbara and Neal Gladstone*
*Lyrics by Kevin Ahern*

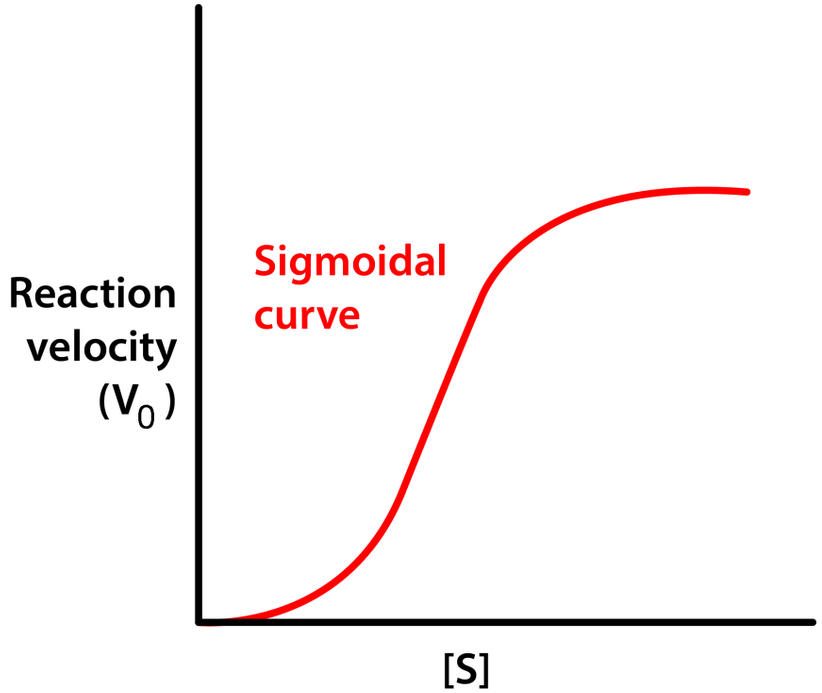
Click HERE, HERE, and HERE for Kevin's Enzyme Regulation lectures on YouTube

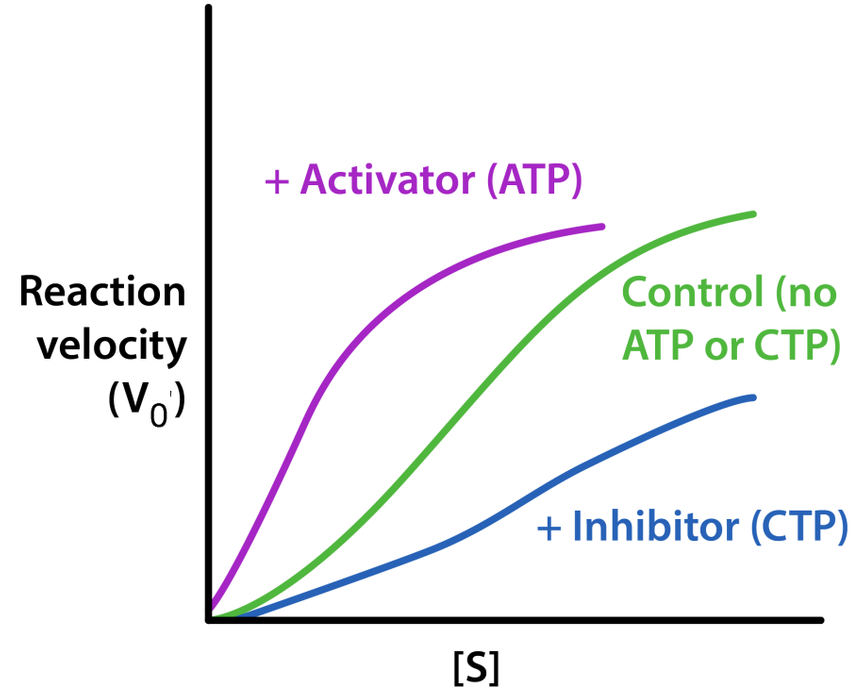
is not a substrate for the enzyme, but, notably, is the end-product of the pathway that HMG-CoA catalyzes a reaction in. When enzymes are inhibited by an end-product of the pathway in which they participate, they are said to be feedback inhibited.

**Feedback inhibition** always operates by allosterism and further, provides important and efficient control of an entire pathway. By inhibiting an early enzyme in a pathway, the flow of materials for the entire pathway is stopped or reduced, assuming there are not alternate supply methods. In the cholesterol biosynthesis pathway, stopping this one enzyme has the effect of shutting off (or at least slowing down) the entire pathway.

Another excellent example is the enzyme **aspartate transcarbamoylase** (**ATCase**), which catalyzes an early reaction in the synthesis of pyrimidine nucleotides. This enzyme has two allosteric effectors, ATP and CTP, that are not substrates and that bind at a regulatory site on the enzyme that is apart from the catalytic, active site. CTP, which is the end-product of the pathway, is a feedback inhibitor of the enzyme. ATP, on the other hand, acts to activate the enzyme when it binds to it.

Allosterically, regulation of these enzymes works by inducing different physical states (shapes, as it were) that affect their ability to bind to substrate. When an enzyme is inhibited by binding an effector, it is converted to the T (also called tight) state, it has a reduced affinity for substrate and it is through this means that the

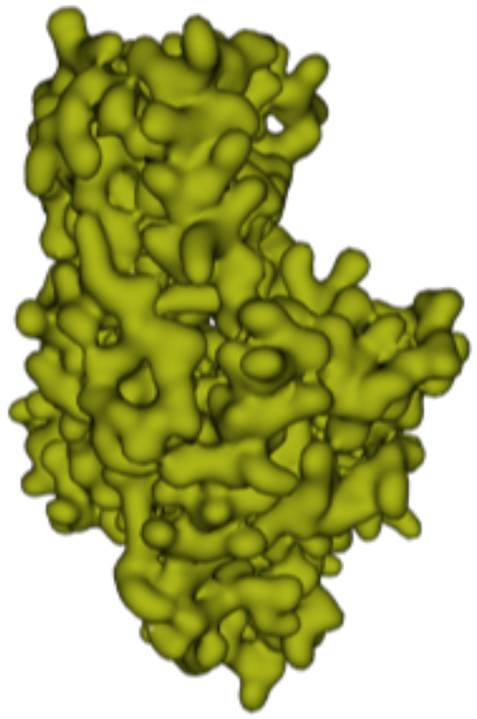



Allosteric Effects on ATCase

reaction is slowed. On the other hand, when an enzyme is activated by effector binding, it converts to the R (relaxed) state and binds substrate much more readily. When no effector is present, the enzyme may be in a mixture of T and R state. The V vs. S plot of allosteric enzymes resembles the oxygen binding curve of hemoglobin (see HERE). Even though hemoglobin is not an enzyme and is thus not catalyzing a reaction, the similarity of the plots is not coincidental. In both cases, the binding of an external molecule is being measured – directly by the hemoglobin plot and indirectly by the enzyme plot, since substrate binding is a factor in enzyme reaction velocity.

**Interactive 4.1**



Hexokinase - Not Bound to Substrate

## Covalent Control of Enzymes

Some enzymes are synthesized in a completely inactive form and their activation requires covalent bonds in them to be cleaved. Such inactive forms of enzymes are called zymogens. Examples include the proteins involved in blood clotting and proteolytic enzymes of the digestive system, such as trypsin, chymotrypsin, and others. The zymogenic forms of these enzymes are known as trypsinogen and chymotrypsinogen, respectively. Synthesizing some enzymes in an inactive form makes very good sense when an enyzme's activity might be harmful to the tissue where they are being made. For example, the painful condition known as pancreatitis arises when digestive enzymes made in the pancreas are activated too soon and end up attacking the pancreas.

Blood clotting involves polymerization of a protein known as fibrin. Since random formation of fibrin is extremely hazardous (heart attack/stroke), the body synthesizes fibrin as a zymogen (fibrinogen) and its activation results from a "cascade" of activations of proteases that arise when a signal is received from a wound. Similarly, removal of fibrin clots is also controlled by a zymogen (plasminogen), since random clot removal would also be hazardous.

Another common mechanism for control of enzyme activity by covalent modification is phosphorylation. The phosphorylation of

enzymes (on the side chains of serine, threonine or tyrosine residues) is carried out by protein kinases. Enzymes activated by phosphorylation can be regulated by the addition of phosphate groups by kinases or their removal by phosphatases.

## Other Enzyme Control Mechanisms

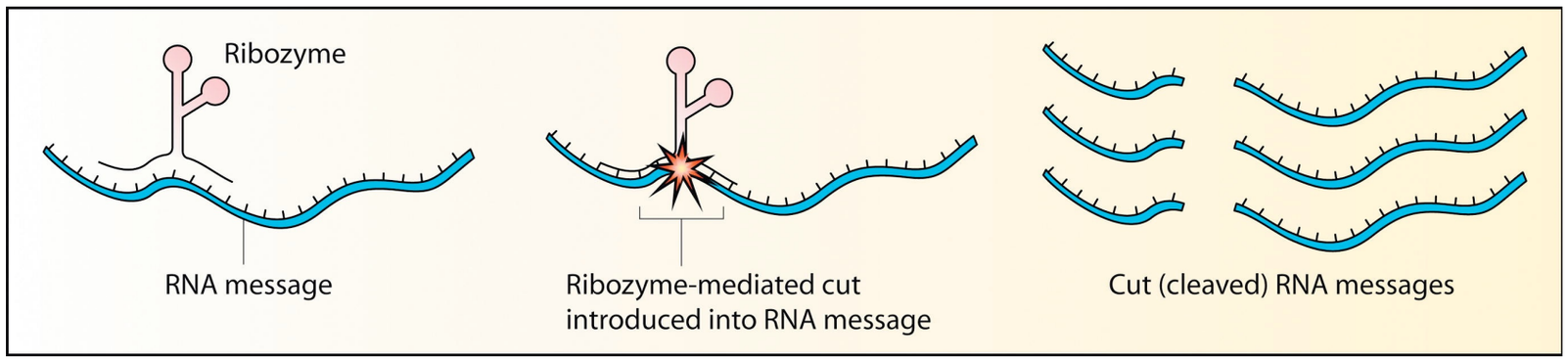
Other means of controlling enzymes relate to access to substrate (substrate-level control) and control of enzyme synthesis. Hexokinase is an enzyme that is largely regulated by availability of its substrate, glucose. When glucose concentration is low, the product of the enzyme's catalysis, glucose-6-phosphate, accumulates and inhibits the enzyme's function.

Regulation of enzymes by controlling their synthesis is covered later in the book in the discussion relating to control of gene expression.

## Ribozymes

Proteins do not have a monopoly on acting as biological catalysts. Certain RNA molecules are also capable of speeding reactions. The most famous of these molecules was discovered by Tom Cech in the early 1980s Studying excision of an intron in *Tetrahymena*, Cech was puzzled at his inability to find any proteins catalyzing the process. Ultimately, the catalysis was recognized as coming from the intron itself. It was a self-splicing RNA and since then, many other examples of catalytic RNAs capable of cutting other RNAs have been found.

Ribozymes, however, are not rarities of nature. The protein-making ribosomes of cells are essentially giant ribozymes. The 23S rRNA of the prokaryotic ribosome and the 28S rRNA of the eukaryotic ribosome catalyze the formation of peptide bonds. Ribozymes are also important in our understanding of the evolution of life on Earth. They have been shown to be capable *via* selection to evolve self-replication. Indeed, ribozymes actually answer a chicken/egg dilemma - which came first, enzymes that do the work of the cell or nucleic acids that carry the information required to produce the enzymes. As both carriers of genetic information and catalysts, ribozymes are likely both the chicken *and* the egg in the origin of life.




**Ribozyme Catalytic Action**

from Wikipedia