K. ENZYME KINETICS AND CATALYSIS

Biochemical reactions are too slow to support life at room temperature. Whether it is the synthesis of proteins, the metabolism of sugars or the oxidation of fatty acids, all of it proceeds on a time scale unsuitable for life. Moreover, many reactions that are undesirable proceed on a comparable timescale, making it even more difficult to coordinate the necessary chemistry to support life. How useful would it be to have polynucleotides degrading faster than they are synthesized?

The basic reactions associated with the synthesis and degradation of essential polymers, and with the metabolism of nutrients all require massive rate accelerations to be of any service to the cell. By specifically accelerating those reactions of interest without accidentally accelerating undesired reactions, as would happen by simply heating the pot, biochemical catalysts shape chemistry in such a way as to permit every biological process required for survival and propagation.

Basic Kinetics

Reaction Rates

The rate of a reaction is defined as the instantaneous rate of change in the concentration of a starting material ("substrate" in biochemical nomenclature – abbreviated "S") or a product with respect to time. By definition, reaction rates are always positive, which leads to the following definition of reaction rate, abbreviated **v** (for velocity) in most of the biochemical literature.

$$S \rightarrow P \qquad \text{(eq. K.1)}$$

$$v = \frac{d[P]}{dt} = -\frac{d[S]}{dt} \qquad \text{(eq. K.2)}$$

Because these are instantaneous rates of change, they can be obtained as tangents to curves that plot [P] or [S] vs. time. Typically, one takes the initial rate (Figure K.1) – as close to the point of initiation of the reaction as possible, since the reaction components are well-defined at that moment. As the reaction progresses the reaction mixture is less well understood, which complicates further analysis of the rate data, as described in the next section.

Rate Laws

The rate of a reaction is typically defined as the rate of production of product with respect to time, d[P]/dt, or **v**, for velocity. That rate can often be defined as proportional to algebraic functions of the reactants, and sometimes products, in a reaction. The proportionality constants are the rate constants. Thus for a reaction in which A + 2B → C, one might arrive at the **rate law**:

$$v = k[A][B] \qquad \text{(eq. K.3)}$$

This rate law is said to be first order in both A and B, and second order overall. The rate constant, written as lower case **k**, is defined therefore as a second order rate constant and will have units of $M^{-1}s^{-1}$, which combines with the units of [A] and [B] to give velocity in M/s. Note that the rate law need not correlate with the stoichiometry, though it may. The stoichiometry will inform the order of the reaction with respect to the reactants if it is an **elementary** reaction, and reflects the explicit interaction of the reactant(s) in a single chemical step. For example, in the simplest of all possible reactions, a single starting reactant, S, reacts independently to create the product P (eq. K.1 above). The rate law for that reaction will be:

$$v = k[S] \qquad \text{(eq. K.4)}$$

Where the reaction is first order and the rate constant is a first order rate constant with units of inverse time.

In both of these examples, the concentration of the reactant(s) will impact the rate of reaction. The more reactant, the faster the rate of reaction, all other things being equal. The other determining influence on reaction rate is the rate constant, whose numerical value is constant really only if everything going on around the reactants is constant: temperature, solvent, ionic strength and so on. Nevertheless, the rate constant is a characteristic of the reaction and can be defined independently of the concentrations of reactants, which are controlled externally, either by the experimenter, or by other cellular processes.

One important caveat. It is difficult to compare first order and second order rate constants to each other. There is often temptation to look at the scalar values, but it is important to remember that the units on a first order rate constant (s^{-1}) and a second order rate constant ($M^{-1}s^{-1}$) are different, so it is something like comparing 1 apple to 2 orangutans, they are different numbers of different beasts.

The Rate Constant and Transition State Theory

You may recall from Chem 102 that the rate of reaction increases with temperature, which led to the formulation of the following equation by Arhennius:

$$k = Ae^{-Ea/RT} \qquad \text{(eq. K.3)}$$

where A is the Arrhenius constant, with the same units as the rate constant, and E_a is the activation energy of the reaction. The exponential term indicates the fraction of molecules possessing the activation energy at a given temperature. As temperature increases, that fraction increases to a maximum value of 1. While this equation works well, it hides the entropic terms in the gemisch that is the Arrhenius constant, and leaves only the activation energy as an approximation of the enthalpic barrier for the reaction.

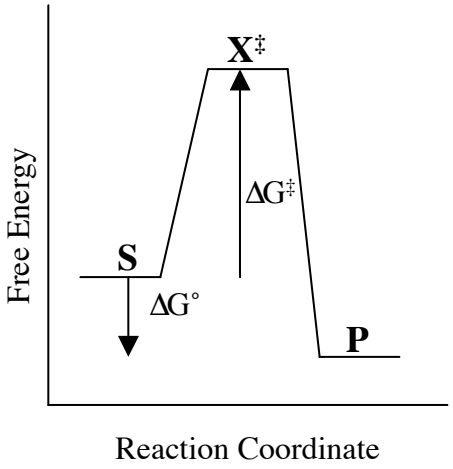

Figure K.1. A reaction coordinate diagram taking starting material S to product P. The equilibrium constant for the reaction is determined by ΔG° and the free energy of activation is $\Delta G^\ddagger$. The transition state is the highest energy structure on the reaction coordinate and is labeled $X^\ddagger$.

Transition state theory allows us to separate the enthalpic and entropic components affecting the rate constant, by replacing the energy of activation with a related term, the **free energy of activation**, $\Delta G^\ddagger$, the free energy barrier to the reaction (Figure K.1). As with any free energy term, we can separate it into the enthalpic and entropic components:

$$\Delta G^\ddagger = \Delta H^\ddagger - T\Delta S^\ddagger \qquad \text{(eq. K.4)}$$

Typically, the formation of $X^\ddagger$ is endothermic, since bonds are typically in the process of breaking, and entropically unfavorable, since you need to organize the starting material(s) just so in order to obtain the reaction. But we'll save that for later. In the meantime, note that the change in free energy of the reaction, ΔG°, allows us to calculate the equilibrium constant for the reaction. The free energy of activation allows us, similarly, to calculate and equilibrium constant – that between the starting material S and the transition state $X^\ddagger$. That equilibrium constant $K^\ddagger$ is defined as:

$$K^\ddagger = \frac{[X^\pm]}{[S]} = \exp\left(-\Delta G^\pm / RT\right) \qquad \text{(eq. K.5)}$$

The larger the value of $\Delta G^\ddagger$, the less favorable the reaction, though by definition, all $\Delta G^\ddagger$ values are positive and thus disfavored. As a result, $X^\ddagger$ is a scarce species. The equilibrium constant does not favor its production, though increasing the temperature will increase the value of $K^\ddagger$ since this is an endothermic reaction.

Inherently, the rate of reaction is determined by how fast $X^\ddagger$ decays to form product. The frequency of breakdown to products is $\nu^\ddagger$ and has a numerical value of 6.2×10^{12} s^{-1} at 298 K.[1] Thus we get the following equation for the rate:

[1] $\nu^\ddagger = k_B T/h$, where k_B is Boltzmann's constant (1.38×10^{-23} J/K) and h is Planck's constant (6.626×10^{-34} Js).

$$v = \nu^{\ddagger}[X^{\ddagger}] = \nu^{\ddagger}K^{\ddagger}[S] \qquad \text{(eq. K.6)}$$

This is a first order rate law, where the rate constant is $\nu^{\ddagger}K^{\ddagger}$. We can substitute for $K^{\ddagger}$ from equation K.5 and obtain the following:

$$k = \nu^{\pm} \exp\left(-\Delta G^{\pm}/RT\right) \qquad \text{(eq. K.7)}$$

Thus we have a form for the rate constant quite similar to the Arrhenius equation, replacing A with $\nu^{\ddagger}$ and E_a with $\Delta G^{\ddagger}$.

Table K.1. Some sample reactions with rate constants at pH 7 and at 298 K.

Reaction	**Rate constant (s^{-1})**	**Half-life (yr)**
$CO_2 + H_2O \rightarrow HCO_3^- + H^+$	0.0358	19 s
Amide hydrolysis	5.4×10^{-11}	400 yr
Phosphodiester hydrolysis	1.7×10^{-13}	130,000 yr

The numerical value of rate constants in biologically interesting reactions is quite variable, ranging from rapid to (literally) deathly slow. A sampling is given in Table K.1. Needless to say, it would be pointless to ingest a steak if the half-life for peptide bond hydrolysis in the body were 400 yr. But just as bad, waiting 20 s for half the CO_2 in the cell to dissolve may be physiologically disastrous for a cell that is undergoing rapid respiration. Similarly, the stability of cellulose would have led to a biological landscape littered with plants that never decay after death, tying up huge quantities of carbon. Clearly nature has developed the means to catalyze those reactions – to raise the rate constants to values that serve the organisms that depend upon them. Catalysis is achieved by lowering the free energy barrier, $\Delta G^{\ddagger}$, thus increasing the population of $X^{\ddagger}$ at a given temperature and enhancing the rate of product formation (Figure K.2).

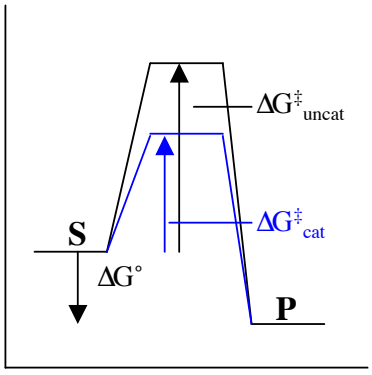

Figure K.2. A reaction coordinate diagram taking starting material S to product P. The equilibrium constant for the reaction is determined by ΔG° and the free energy of activation is $\Delta G^{\ddagger}$. The transition state is the highest energy structure on the reaction coordinate and is labeled $X^{\ddagger}$.

In solution, one can often lower the free energy of the transition state simply by changing solution conditions. For example, S_N2 nucleophilic displacement reactions are typically faster in polar aprotic solvents, since the negative charge build-up on the leaving group and positive charge building up on the nucleophile can both be stabilized by interactions with the polar medium.[2] Since most biochemical reactants take place in a common environment, the cytoplasm of a cell, there's not much hope of optimizing the rate of reaction by tailoring the solvent conditions. Instead, biochemical catalysis is achieved by creating a specialized environment for each reaction that selectively complements and stabilizes the transition state of that one reaction and none other. That specialized environment is the active site of an enzyme.

Michaelis-Menten Kinetics

Enzymes

For every biochemical reaction requiring acceleration, there is an enzyme – a molecule, typically a protein though sometimes RNA, that acts as a catalyst. While some enzymes are generalists, acting on many related reactants, many are quite specialized, focusing on a single reaction. The middle part of the 20th century saw a huge investment in discovering new enzyme activities and categorizing them. The recent advent of whole genome sequencing continues to add new activities, and the increasing appreciation of the biodiversity of microbes has likewise expanded our understanding of the range of catalysts operating under a variety of unusual conditions enabling a suite of potentially useful transformations. Enzyme activities are categorized by **Enzyme Commission Number**, which provides a sorting mechanism for the diversity of catalysts that have been identified. At the highest level, there are six classes of enzyme activities as listed in Table K.2.

Table K.2. Listing of Enzyme Commission categories

E.C. Category	Reaction Class	Example
1	Oxidoreductases	Alcohol dehydrogenase oxidizes ethanol to acetaldehyde.
2	Transferases	EcoR I methylase transfers a methyl group from S-adenosyl methionine to the N2 amino group of adenine.
3	Hydrolases	Trypsin hydrolyzes the peptide bond in proteins
4	Lyases	Aldolase breaks the carbon-carbon bond of fructose-1,6-bisphosphate.
5	Isomerases	Xylose isomerase interconverts the aldose xylose with the ketose xylulose, both $C_5H_{10}O_5$.
6	Ligases	Aminoacyl-tRNA synthetases create covalent connections between amino acids and tRNA molecules.

[2] Conversely, the rate can be slowed in polar, *protic*, solvents like alcohols because the nucleophile becomes stablilized by interactions with the solvent, effectively lowering the energy of S and putting a steric barrier of desolvation that must be overcome before the nucleophile can react.

Typically an enzyme's name reflects its function, though many archaic names are still out there. For example trypsin tells you nothing, but describing it as an endopeptidase tells you that it cleaves proteins at internal peptide bonds (the suffix *–ase* is common among all IUPAC-approved enzyme names).

A Simple Rate Law for an Enzyme-Catalyzed Reaction

Enzyme reaction mechanisms can be quite complex and involve several steps that must each be considered in proposing a rate law that fully describes catalytic behavior. In this section, we will focus on the simplest of all possible enzyme catalyzed reactions, in which a single substrate is converted to a single product in the presence of an enzyme:

$$E + S \rightarrow E + P \qquad \text{(eq. K.8)}$$

Early in the last century it was recognized that this is not a simple, one-step mechanism. Were it so, the predicted rate law for the reaction would have the a "molecularity" that reflects the stoichiometry of the reaction. The rate law would be first order in enzyme and in substrate:

$$v = k[E][S] \qquad \text{(eq. K.9)}$$

Thus, one would predict that there would be a linear increase in the initial velocity of a reaction as one raised substrate concentration while holding the concentration of enzyme constant (Figure K.3). In fact, that is not the case. One instead observes **saturation** behavior. The rate of reaction levels off to some maximal rate at high substrate concentration (Figure K.3).

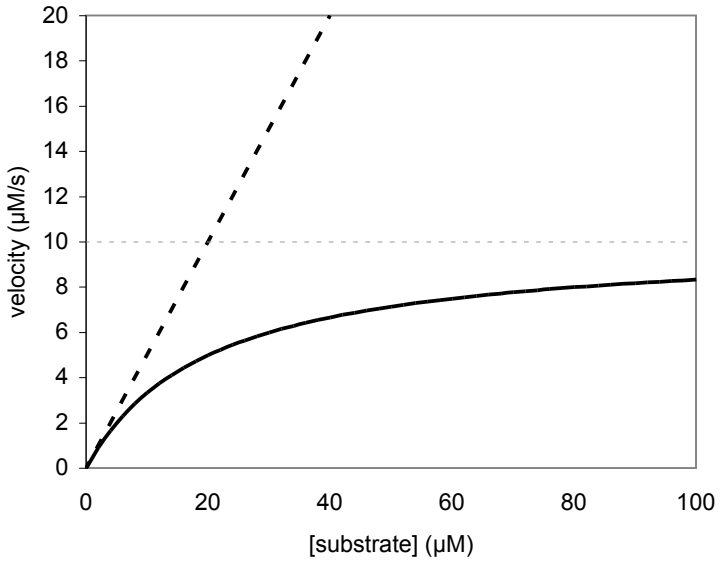

Figure K.3. The rate relationship to substrate concentration in enzyme catalyzed reactions. A first order reaction in substrate would give a straight line (dashed), but enzyme-catalyzed reactions typically show saturation behavior and reach a maximum velocity at high substrate concentration (solid line). Here, the maximal velocity (V_{max}) is 10 μM/s, and is indicated by a faint horizontal line.

Saturation kinetics can be explained by the following mechanism, first sketched out by Michaelis and Menten in 1912 (and often called the Michaelis-Menten mechanism):

$$E + S \leftrightarrows E\bullet S \rightarrow E + P \qquad \text{(eq. K.10)}$$

The reaction takes place in two discrete steps. The first involves reversible formation of a non-covalent complex between the enzyme and substrate (E•S), sometimes called the Michaelis complex, and the second step involves breakdown of the E•S complex to form product, and to regenerate the free enzyme. A rate law may be predicted from this mechanism, by employing the **steady state hypothesis**, which argues that during the initial period of a reaction, the concentration of the intermediates in the reaction will remain roughly constant.

The derivation of a rate law by the steady state hypothesis proceeds as follows. First, one notes that the rate of product formation can be no faster than the rate of breakdown of the E•S complex. We assign a rate constant to that step, k_{cat}:

$$E \bullet S \xrightarrow{k_{cat}} E + P \qquad \text{(eq. K.11)}$$

That indicates a rate law:

$$v = k_{cat}[E\bullet S] \qquad \text{(eq. K.12)}$$

This is a valid rate law, except that the concentration of the E•S complex will generally be unknown to us, since it is not a compound whose concentration is measured out into solution. The compounds whose initial concentration that we *do know* are the enzyme and substrate. Thus the goal of the steady state hypothesis is to express the concentration of the E•S complex as a function of [E] and [S]. To do that, first let's assign two other microscopic rate constants to the reaction:

$$E + S \xrightarrow{k_1} E \bullet S$$
$$E \bullet S \xrightarrow{k_{-1}} E + S \qquad \text{(eq. K.13)}$$

This essentially defines a rate constants for the reversible first step of the Michaelis-Menten equation. In so doing, we can obtain rate laws for the consumption and formation of the E•S complex.

$$v_{formation} = k_1[E][S] \qquad \text{(eq. K.14)}$$

$$v_{consumption} = k_{-1}[E\bullet S] + k_{cat}[E\bullet S] \qquad \text{(eq. K.15)}$$

The steady state hypothesis argues that the concentration of E•S is constant over the initial period of a reaction. That means:

$$v_{formation} = v_{consumption} \qquad \text{(eq. K.16)}$$

or:

$$k_{-1}[E\bullet S] + k_{cat}[E\bullet S] = k_1[E][S] \qquad \text{(eq. K.17)}$$

Solving for the concentration of E•S, we obtain:

$$[E \bullet S] = \frac{k_1[E][S]}{k_{-1} + k_{cat}} \qquad \text{(eq. K.18)}$$

To simplify this equation, we define the **Michaelis constant**, K_m, as follows:

$$K_m = \frac{k_{-1} + k_{cat}}{k_1} \qquad \text{(eq. K.19)}$$

Note that this is **not an equilibrium constant**, but it is a convenient assemblage of rate constants, that allows us to express the concentration of E•S in terms of [E] and [S].

$$[E \bullet S] = \frac{[E][S]}{K_m} \qquad \text{(eq. K.20)}$$

Now we may provide a rate law for the mechanism in equation K.10. Using equations K.12 and K.20, we obtain:

$$v = \left(\frac{k_{cat}}{K_m}\right)[E][S] \qquad \text{(eq. K. 21)}$$

Note that this predicts a second order rate law just as we saw in equation K.9. But not really, because the concentration of enzyme in equation K.21 is *free enzyme*, which is only a fraction of the enzyme present in solution if some of the enzyme is bound up as E•S. Thus we need to adapt the rate law once further. We need to solve for rate in terms of *total enzyme concentration*, $[E]_{tot}$, and substrate concentration.

$$\frac{[E \bullet S]}{[E]_{tot}} = \frac{[E \bullet S]}{[E] + [E \bullet S]} \qquad \text{(eq. K.22)}$$

Here we are distinguishing between free and bound enzyme. Substituting from equation K.20, we get:

$$\frac{[E \bullet S]}{[E]_{tot}} = \frac{[E][S] \big/ K_m}{[E] + [E][S] \big/ K_m} = \frac{[S]}{K_m + [S]} \qquad \text{(eq. K.23)}$$

Which allows us to substitute in a different way for [E•S] in equation K.12:

$$v = \frac{k_{cat}[E]_{tot}[S]}{K_m + [S]} \qquad \text{(eq. K.24)}$$

This is the so-called Michaelis-Menten equation (though really Briggs and Haldane are responsible for applying the steady state assumption). Remember this equation, and you too may be hired to teach biochemistry some day!

In any event, the Michaelis-Menten equation successfully predicts the saturation behavior observed in Figure K.3. As [S] grows to large values, it overwhelms the value of K_m in the denominator and one obtains:

$$V_{max} = k_{cat}[E]_{tot} \qquad \text{(eq. K.25)}$$

What this says, is that $v = V_{max}$ when $[E\bullet S] = [E]_{tot}$. *Maximal velocity is obtained when all of the enzyme is saturated with substrate*; there is no free enzyme left in solution. This represents an absolute barrier to the rate of reaction.

Note that we now have four constants that arise from the Michealis-Menten equation: V_{max}, k_{cat}, K_m and more obliquely, k_{cat}/K_m. Each of these has important interpretations associated with it in the analysis of enzyme behavior, and each will be addressed below.

The Meaning of V_{max}

Well, actually, this has already been given. It is the maximum rate that can be achieved by an enzyme-catalyzed reaction, *at a given concentration of enzyme*. V_{max} is not a constant for the reaction, but rather is a variable that is obtained under certain reaction conditions. Nevertheless, it is important to remember equation K.25 above and its implication of saturation behavior.

The Meaning of K_m

K_m is a tricky kinetic constant. It is **not an equilbrium constant**, but it looks just like one, what with being a capital "K" and having the following relationship:

$$K_m = \frac{[E][S]}{[E \bullet S]} \qquad \text{(eq. K.26)}$$

This looks just like a ligand dissociation constant, but it isn't… quite. It gives the balance of free enzyme and substrate vs. E•S complex under the conditions of the steady state. But – there are circumstances in which K_m *approximately* equals the dissociation constant for the substrate, K_d. When k_{cat} is much, much smaller than the rate constant for dissociation of the substrate from the enzyme without any reaction, k_{-1}, we obtain from equation K.19:

$$K_m = \frac{k_{-1} + k_{cat}}{k_1} \approx \frac{k_{-1}}{k_1} = K_d \qquad \text{(eq. K.27)}$$

Note that this is an *approximation* and that italics are appearing all over the place here to warn you not to be too ready to interpret K_m as an equilibrium constant. But often, as we'll see, k_{cat} is smaller than k_{-1} and equation K.27 holds.

Graphically speaking, there is another interpretation of K_m, which is similar to the graphical interpretation of K_d. K_m is the substrate concentration that gives a velocity equal to one-half of V_{max}:

$v = {}^1/_2 V_{max}$ when $[S] = K_m$

To see why, revisit the Michaelis-Menten equation (eq. K.24). When [S] = K_m

$$v = \frac{k_{cat}[E]_{tot}K_m}{K_m + K_m} = \frac{1}{2}k_{cat}[E]_{tot} \qquad \text{(eq. K.28)}$$

This means that the graph in Figure K.3 can be used to estimate K_m. Note that V_{max} is 0.01 μM/s in that plot. What concentration of substrate gives a velocity of 0.005 μM/s?[3]

Alan Fersht has used this relationship to argue that a well-optimized enzyme will be tuned to have a K_m value for its substrate that roughly matches the concentration of substrate present in the cell. Why? If K_m were much smaller than the concentration of substrate, then the enzyme would be saturated under all conditions. An influx of some metabolite would thus find no catalyst available to help convert it. An enzyme working at maximum velocity is essentially falling behind in its work. Adding more work would be problematic for the cell. Similarly, if K_m is much higher than the cellular concentration of a substrate, most of the enzyme is in its free state. That is inefficient because most of the enzyme is being left idle, a waste of resources. In comparing K_m values for enzymes with substrate concentrations, Fersht has been able to show an approximate relationship (Table K.3).

Table K.3. Comparison of K_m values and substrate concentrations for some common enzymes in the cell.[4]

Enzyme	**Substrate**	**[S] (μM)**	**K_m (μM)**
Glucose-6-phosphate isomerase	Glucose-6-phosphate	130	210
Lactate dehydrogenase	Pyruvate	51	59
Aldolase	Fructose-1,6-bisphosphate	32	100

In the future, you will often be tempted to use K_m as a measure of how tightly a substrate binds to an enzyme active site. Sometimes that will be OK, but remember that the approximation only holds when k_{cat} is much less than k_{-1}.

The Meaning of k_{cat} – the Turnover Number

The catalytic rate constant, k_{cat}, is a first order rate constant that gives the frequency of decomposition of the E•S complex to products. Note that its units are inverse time. Sometimes k_{cat} is referred to as the **turnover number**. Because V_{max} is obtained by equation K.25, one can think of k_{cat} as the frequency with which an enzyme, operating at saturation, can convert substrate to product – how frequently the enzyme turns over. This can be very fast, as it turns

[3] The answer? K_m is 2 μM.

[4] A. Fersht, *Structure and Mechanism in Protein Science*, Freeman, 1999, pp.. 366-367. Note that I picked some of the better examples for illustrative purposes!

out. Some examples are given in Table K.4 along with the rate constants for the uncatalyzed reactions.

The comparison of k_{cat} to k_{uncat} is a common one, because it compares two rate constants of the same molecularity (ie. usually both are first order rate constants). The explicit comparison is as follows (and is diagrammed in Figure K.4):

$$S \xrightarrow{k_{uncat}} P$$
$$E \bullet S \xrightarrow{k_{cat}} E + P \qquad \text{(eq. K.29)}$$

This comparison provides a useful measure of how much the enzyme is capable of decreasing $\Delta G^{\ddagger}$ for a given reaction, by allowing that reaction to take place *inside the enzyme active site.* Hence the distinction in Figure K.4 between E•S and E+S. The active site on an enzyme is usually a pocket on the surface of the protein into which the substrate is bound. This environment is distinct from bulk water, and is designed to either selectively destabilize the substrate with respect to the transition state, or to stabilize the transition state with respect to the substrate. Changing solvents can affect the rate of a reaction, and it might be helpful to think of the active site as simply a specialized solvent environment for the moment.

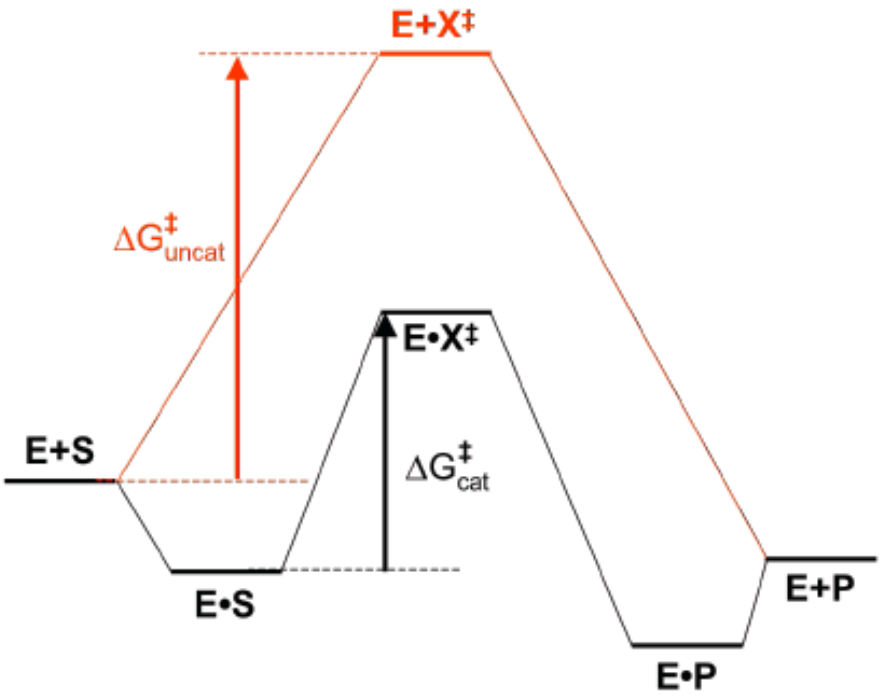

Figure K.4. Free energy diagram relating the free energy of activation for the uncatalyzed process vs. the catalyzed process. The $\Delta\Delta G^{\ddagger}$ is the difference in the heights of these two arrows. Note that a large $\Delta G^{\ddagger}$ reflects a small rate constant.

From a comparison of k_{cat} and k_{uncat}, one can obtain a difference in the free energy of activation, $\Delta\Delta G^{\ddagger}$ (see Figure K.4):

$$\Delta\Delta G^{\ddagger} = -RT\ln(k_{cat}/k_{uncat}) \qquad \text{(eq. K.30)}$$

Where the change in the magnitude of $\Delta G^{\ddagger}$ is determined relative to the uncatalyzed rate constant (the reference in this instance). Some examples are provided in Table K.4.

Table K.4 Some comparative values for k_{cat} and k_{uncat} and the difference in free energy of activation of the catalyzed reaction relative to the uncatalyzed reaction.

Enzyme	**k_{cat} (s^{-1})**	**k_{uncat} (s^{-1})**	**$\Delta\Delta G^{\ddagger}$ ($^{kcal}/_{mol}$)**
Carbonic anhydrase	10^6	0.13	-9.5
Cocaine esterase	7.8	1.7×10^{-6}	-9.1
Orotate decarboxylase	39	2.8×10^{-16}	-23.4

It is important to note, however, that looking at k_{cat} alone is a poor way to describe the catalytic power of an enzyme. It presupposes that the reaction starts with the E•S complex, and completely neglects the impact of substrate binding. A more complete analysis must include the k_1 and k_{-1} rate constants.

The Meaning of k_{cat}/K_m – the Specificity/Efficiency Constant

Recall equation equation K.21 from above, placed here for your viewing convenience:

$$v = \left(\frac{k_{cat}}{K_m}\right)[E][S] \qquad \text{(eq. K. 21)}$$

This is a second order rate law that describes the rate of reaction between free enzyme and substrate to make enzyme and product, as described in equation K.8 (here again with a rate constant associated):

$$E + S \xrightarrow{k_{cat}/K_m} E + P \qquad \text{(eq. K.30)}$$

Note that previously, we disallowed further consideration of equation K.21 because it depends on the concentration of a species whose concentration may actually unknown to us – that of the free enzyme. But under conditions of low substrate conentration, most enzyme is free enzyme, so we can approximate [E] as $[E]_{tot}$ and equation K.21 is easy to apply. Essentially k_{cat}/K_m describes how effective the catalyst is at converting free substrate to free product – the overall rate of the reaction. As a result, k_{cat}/K_m may be called the **overall rate constant**, or more commonly the **efficiency constant**, since it reports on how efficiently S is converted to P (rather than ES to EP, as k_{cat} does).

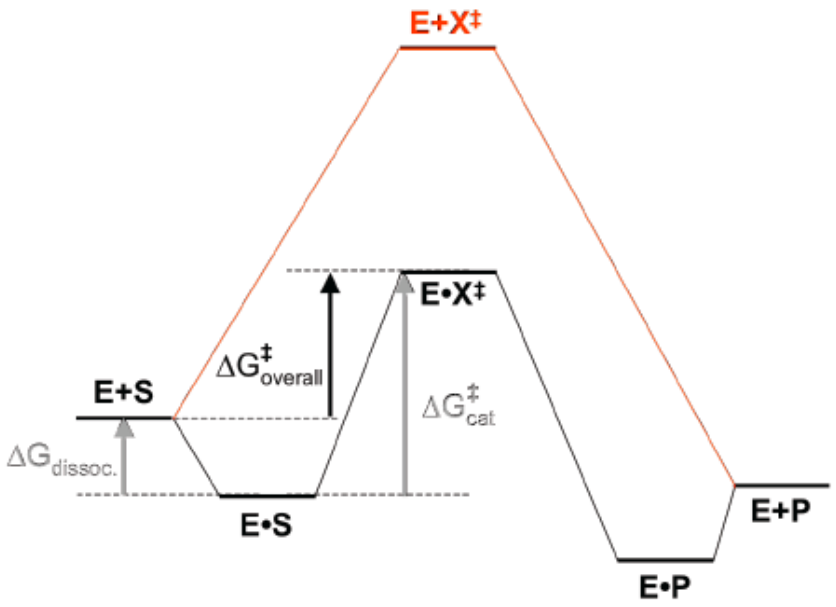

Figure K.5. $\Delta G_{overall}^{\ddagger}$ is the difference in free energy from the E+S state to the highest point on the reaction coordinate ($E{\bullet}X^{\ddagger}$ here). Note that it can be obtained by subtracting the free energy of dissociation of the E•S complex from the free energy of activation of the catalyzed process.

Graphically, k_{cat}/K_m is related to the free energy barrier that lies between free substrate and product ($\Delta G_{overall}^{\ddagger}$). In cases where the catalytic step is rate limiting (that is when $\Delta G_{cat}^{\ddagger}$ is larger than the barriers for loading substrate onto the enzyme or releasing it from the enzyme), then the free energy barrier is measured from the E+S state to the $E{\bullet}X^{\ddagger}$ state (Figure K.5). Note that a relationship exists between $\Delta G_{overall}^{\ddagger}$ and the $\Delta G_{cat}^{\ddagger}$ and the free energy of dissociation of the E•S complex:

$$\Delta G_{overall}^{\ddagger} = \Delta G_{cat}^{\ddagger} - \Delta G_{dissoc.} \qquad \text{(Eq. K.31)}$$

In cases where the catalytic step is rate limiting, K_m approximates the dissociation constant, so the following derivation holds:

$$-\Delta G^{\pm}_{overall} \Big/ RT = -(\Delta G^{\pm}_{cat} - \Delta G_{dissoc.}) \Big/ RT$$

$$\exp\left[-\Delta G^{\pm}_{overall} \Big/ RT\right] = \exp\left[-(\Delta G^{\pm}_{cat} - \Delta G_{dissoc.}) \Big/ RT\right]$$

$$\exp\left[-\Delta G^{\pm}_{overall} \Big/ RT\right] = \exp\left[-\Delta G^{\pm}_{cat} \Big/ RT\right] \Big/ \exp\left[-\Delta G_{dissoc} \Big/ RT\right] \quad \text{(Eq. K.32 \& then some)}$$

$$k_{overall} \Big/ \nu^{\pm} = \left(k_{cat} \Big/ \nu^{\pm}\right) \Big/ K_d$$

$$k_{overall} = k_{cat} \Big/ K_d = k_{cat} \Big/ K_m$$

It's generally useful to recognize that addition and subtraction of free energies correspond to multiplication and division of rate and equilibrium constants.

One way in which to appreciate the magnitude of k_{cat}/K_m values of enzymes is to compare them to diffusion rate constants – how fast do two molecules collide with each other. Certainly, one wouldn't expect enzyme reactions to take place more rapidly than the rate of diffusion, which is on the order of 10^8-10^9 $M^{-1}s^{-1}$ for a general enzyme-catalyzed reaction. In some instances this rate is nearly met. For example, carbonic anhydrase, which catalyzes the reaction of CO_2 with water:

$$CO_2 + H_2O \rightarrow HCO_3^- + HO^- \qquad \text{(eq. K.33)}$$

Performs the chemistry with a k_{cat} of 1.0 x 10^6 s^{-1} and a K_m of 8 mM for CO_2. The efficiency constant is 1.3 x 10^8 $M^{-1}s^{-1}$, clearly close to the diffusion limit. In general, values of k_{cat}/K_m range upwards of 10^5 $M^{-1}s^{-1}$, reflecting the extraordinary catalytic efficiency of enzymes.

Another useful application of k_{cat}/K_m is in comparing the **efficiency** of enzymes with multiple substrates. Neither k_{cat} nor K_m are suitable individually in comparisons of catalytic function since the former neglects the key substrate binding step and the latter neglects catalysis. Only k_{cat}/K_m considers both how well the substrate is bound and how rapidly it is converted from that point on to product. So, consider an enzyme that catalyzes the tranformation of either substrate A or substrate B to products:

$$E + A \xrightarrow{(k_{cat}/K_m)_A} E + P_1$$
$$E + B \xrightarrow{(k_{cat}/K_m)_B} E + P_2 \qquad \text{(Eq. K.34)}$$

The relative rates of reaction, assuming that the concentrations of both substrates are equal, is as follows:

$$\frac{v_A}{v_B} = \frac{\left(k_{cat}/K_m\right)_A [A][E]}{\left(k_{cat}/K_m\right)_B [B][E]} = \frac{\left(k_{cat}/K_m\right)_A}{\left(k_{cat}/K_m\right)_B} \qquad \text{(Eq. K.35)}$$

The Meaning of $k_{uncat}/(k_{cat}/K_m)$ – The Proficiency Constant[5]

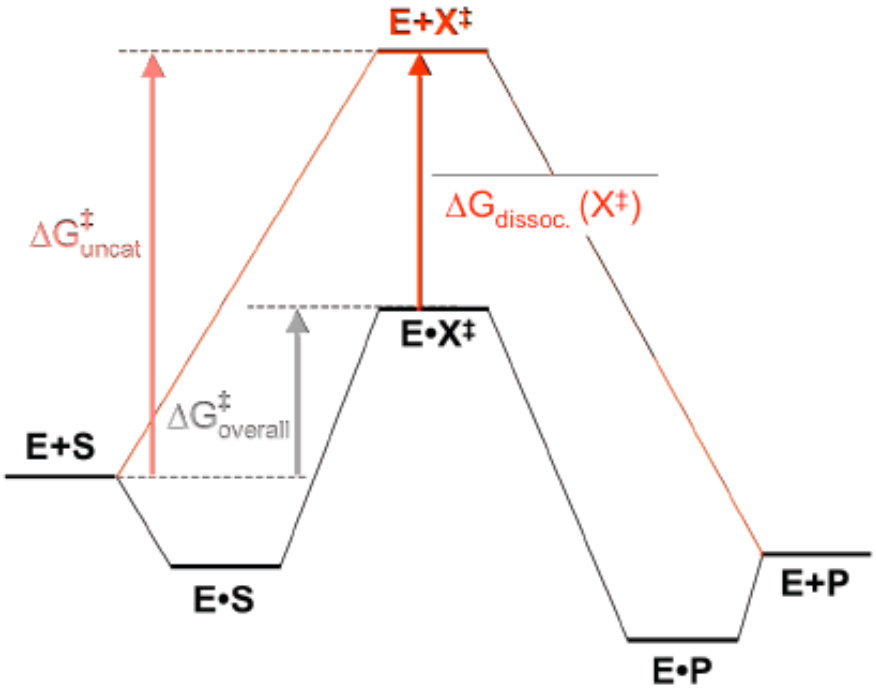

Figure K.6. Schematic showing how the $\Delta G_{dissoc.}$ of $X^{\ddagger}$ is related to the free energies associated with the catalytic barrier of the uncatalyzed process and of the overall catalyzed process.

So, finally, how good a catalyst is an enzyme? Is carbonic anhydrase the greatest enzyme around because it catalyzes its reaction at the diffusion limit, or should we identify enzymes that do the best job of stabilizing the transition state (looking at $\Delta\Delta G^{\ddagger}$), neglecting the role of substrate binding in catalysis? The proficiency constant, $k_{uncat}/(k_{cat}/K_m)$, provides a comparison that gives an absolute value for the binding of the transition state by the enzyme, allowing side-by-side comparison of enzymes working on both difficult and easy-to-catalyze reactions. From Figure K.6, it can be seen that the proficiency constant is equivalent to the dissociation constant of $X^{\ddagger}$ from the enzyme active site. By analogy to the meaning of k_{cat}/K_m, we can see from Figure K.6 that:

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[5] I'm taking this notion from the review by Wolfenden (2001) [*Acc. Chem. Res.* **34**, 938-945], but with a slight misappropriation – he uses the inverse of the value I cite as an association constant. I like it better as a dissociation constant. In either form, it is not as broadly used (yet) as k_{cat}/K_m but clearly speaks to a fundamental aspect of enzymatic catalysis.

$$\Delta G_{dissoc.}(X^{\ddagger}) = \Delta G_{uncat}^{\ddagger} - \Delta G_{overall}^{\ddagger} \qquad \text{(Eq. K.36)}$$

so that:

$$K_d\,(X^{\ddagger}) = k_{uncat}/k_{overall} = k_{uncat}/(k_{cat}/K_m) \qquad \text{(Eq. K.37)}$$

In this comparison, carbonic anhydrase is seen (Table K.6) to have a respectable proficiency constant, at 10^{-9} M (that is, the enzyme binds the transition state with nanomolar affinity), but orotate decarboxylase, with a k_{uncat} of roughly 10^{-15} s^{-1} and a k_{cat}/K_m of roughly 10^8 $M^{-1}s^{-1}$ has a proficiency constant of $\sim 10^{-23}$ M. Now that is some real binding going on there!

Table K.6. Examples of proficiency constants for several enzymes.[6]

Enzyme	k_{uncat} (s^{-1})	k_{cat} (s^{-1})	K_m (M)	k_{cat}/K_m ($M^{-1}s^{-1}$)	$k_{uncat}/(k_{cat}/K_m)$ (M)
Carbonic anhydrase	0.13	1×10^6	0.008	1.3×10^8	1×10^{-9}
Orotate decarboxylase	3.0×10^{-16}	39	7.0×10^{-7}	5.6×10^7	5×10^{-24}
Cytidine deaminase	3.2×10^{-10}	299	1.0×10^{-4}	2.9×10^6	1.1×10^{-16}

Inhibition

There are substrates and there are products, and then there are **inhibitors** – molecules that act to block the progress from S to P. These compounds are of significant interest in the pharmaceutical industry since they are capable of blocking undesirable physiological processes – such as cholesterol biosynthesis, bacterial cell wall synthesis, retroviral propagation and the like. More generally, inhibitors can reveal details of enzyme structure/function relationships.

Types of Inhibition

There are three general mechanisms of reversible inhibition that apply to Michaelis-Menten kinetics: **competitive**, **non-competitive** and **uncompetitive** (Figure K.7). Competitive inhibition receives its name due to the competition between the substrate and the inhibitor for a common binding site. In non-competitive inhibition, the inhibitor binds equally well to both the free enzyme and the enzyme-substrate complex, obviously at a site distinct from the substrate binding site. Non-competitive inhibitors work via allostery, blocking enzyme action from a distance. Uncompetitive inhibition is relatively rare and usually only is found in enzymes that bind two substrates (so that the uncompetitive inhibitor competes out the second substrate). A further class of inhibitors are the so-called **mixed** inhibitors. Like non-competitive inhibitors, they bind to both the free enzyme and E•S complex, but with different equilibrium constants.

[6] Data taken from Radzicka & Wolfenden (1995) *Science* **267**, 90-93.

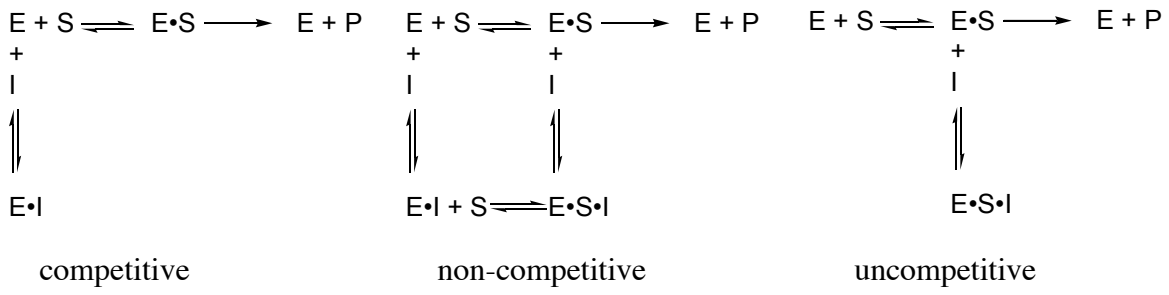

Figure K.7. Three mechanisms of enzyme inhibition.

The Kinetics of Competitive Inhibition

In competitive inhibition, the inhibitor binds reversibly to the enzyme, yielding a dissociation constant, K_i (Eq. K.38).

$$E\bullet I \Leftrightarrow E + I \qquad K_i = \frac{[E][I]}{[E \bullet I]} \qquad \text{(Eq. K.38)}$$

To determine the effect of the inhibitor on the rate of an enzymatic reaction, we need to revisit the set-up described in equation K.22, but modify it, recognizing that the enzyme is partitioned between three separate species in solution when both substrate and inhibitor are present.

$$\frac{[E \bullet S]}{[E]_{tot}} = \frac{[E \bullet S]}{[E] + [E \bullet S] + [E \bullet I]} \qquad \text{(Eq. K.39)}$$

Subsituting for [E•S] and [E•I], the derivation of the rate law proceeds as follows.

$$\frac{[E \bullet S]}{[E]_{tot}} = \frac{[E][S]/K_m}{[E] + [E][S]/K_m + [E][I]/K_i} \qquad \text{(Eq. K.40 and more)}$$

$$\frac{[E \bullet S]}{[E]_{tot}} = \frac{[S]}{K_m + [S] + K_m[I]/K_i} = \frac{[S]}{K_m\left(1 + [I]/K_i\right) + [S]}$$

The parenthetical term $(1+[I]/K_i)$ is often substituted with α for simplicity sake, but I'll leave it alone here. Again, since the rate of reaction is:

$$v = k_{cat}[E\bullet S]$$

We get

$$v = \frac{k_{cat}[E]_{tot}[S]}{K_m\left(1 + [I]/K_i\right) + [S]} \qquad \text{(Eq. K.41)}$$

which describes the effect of an inhibitor on the rate of reaction. As [I] increases, the effect will be to reduce the rate of reaction at any given substrate concentration. However, note carefully

that it is still possible to saturate the enzyme with substrate. It just requires more substrate to saturate than when no inhibitor is present (Figure K.8).

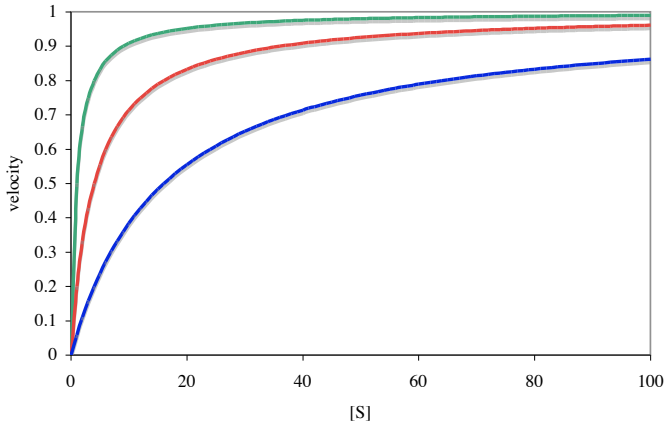

Figure K.8. Effect of a competitive inhibitor on rate vs. [S] profiles. The addition of the inhibitor ([I] increases from green to red to blue) causes an increase in the amount of substrate required to reach 50% of V_{max}, but does not change the value of V_{max} itself.

Transition State Analogs as Competitive Inhibitors

As noted above, competitive inhibitors are often excellent tools for probing the structure of enzyme active sites, identifying important interactions necessary to catalysis. Recall that enzymes have evolved to selectively bind the transition state of a desired reaction relative to the substrate, thus lowering the free energy of activation. Thus, molecules that look a great deal like the transition state often make the best inhibitors. We will revisit that point in several enzyme-specific discussions to come, but it's worth mentioning a couple of classic cases.

Cytidine deaminase catalyzes the exchange of a water for ammonia at C4 of the cytosine base in its nucleoside, proceeding through a tetrahedral intermediate to form uridine (Figure K.9). An inhibitor with an sp^3 center at C4 proves to bind strongly to the active site (K_i = 400 nM) while the substrate only binds with a dissociation constant of roughly 100 μM (based on K_m). Presumably the tighter binding is thanks to selective binding of the tetrahedral conformation at C4.

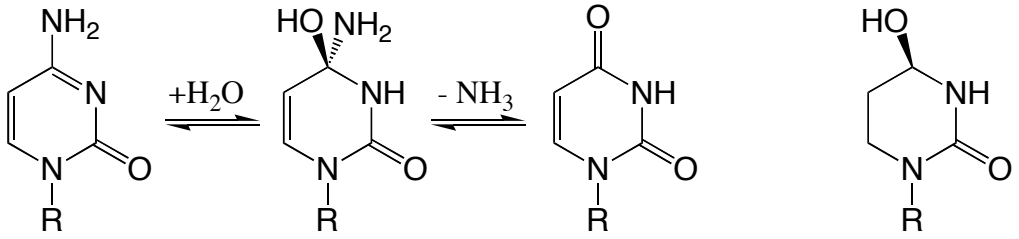

Figure K.9. Reaction catalyzed by cytidine deaminase. At right is a transition state analog. It binds with a K_i of 400 nM.

Proline racemase is another classic case of inhibition by a transition state analog. The enzyme operates by deprotonating the α carbon, leading to an sp^2 center at that carbon. Inhibitors that mimic that planar structure bind more tightly to the enzyme that the substrate does (Figure K.10). The K_i of the pyrrole is 14 μM, while the dissociation constant for proline (estimated from the K_m) is roughly 2 mM.

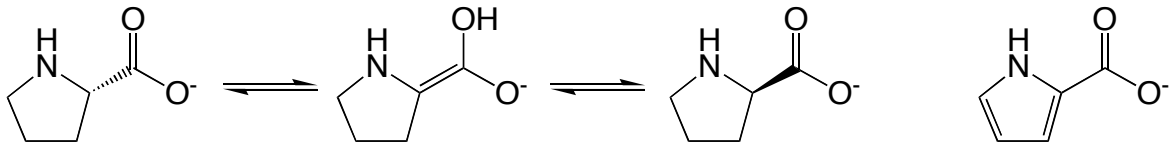

Figure K.10. Reaction catalyzed by proline racemase. The sp^2 center of the high energy intermediate is mimicked by the pyrrole ring at right. That compound acts as a competitive inhibitor with a K_i of 14 μM.