# CHEMICAL KINETICS II. TEMPERATURE DEPENDENCE, TRANSITION STATE THEORY, KINETIC LIMITS, AND ENZYMATIC REACTIONS

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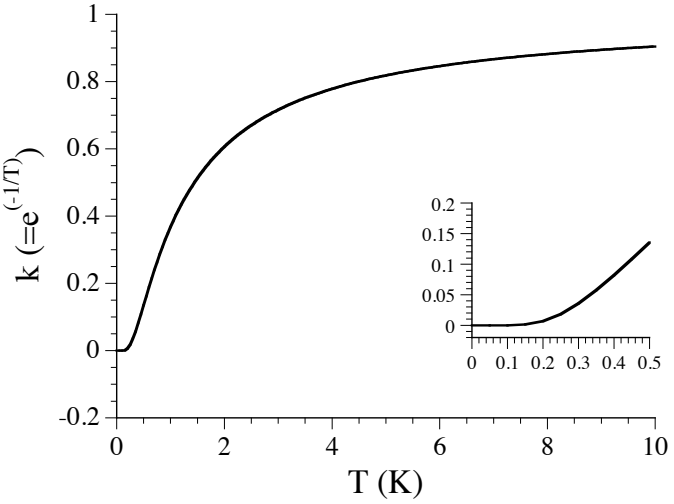
## 1. The temperature dependence of chemical reactions.

***The Arrhenius Equation.*** For many reactions, rates increase with increasing temperature. For simple chemical reactions, a reasonable rule of thumb is that the rate will increase somewhere between two- and and four-fold for a ten degree (C or K) increase in temperature. A more analytical description is referred to as the Arrhenius equation:

$$k_{obs} = Ae^{(-E_a / RT)}$$

The term *A*, a *pre-factor*, is found to be (nearly) temperature independent. *A* has units consistent with the rate constant: $sec^{-1}$ for a first-order rate constant, $M^{-1}sec^{-1}$ for a second order rate constant. $E_a$ is called the activation energy, and is often thought of as the threshold energy for the reaction. The dimension of $E_a$ is energy; the units are determined by those of *R* (kcal•mol$^{-1}$•K$^{-1}$, J•mol$^{-1}$•K$^{-1}$). T is in Kelvins. A plot of the Arrhenius equation is shown below:

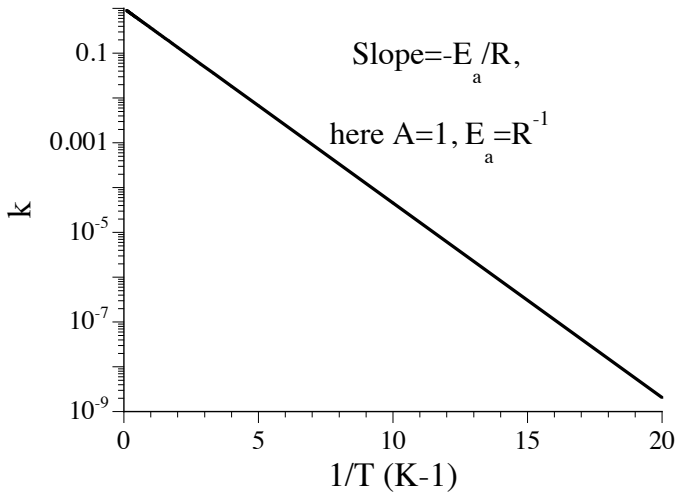



To make things simple in this plot, *A* was set 1, and $E_a$ was set to *R* times $1K^1$; this leads to the simple relation $k_{obs}=e^{-1/T}$ (this would be a very low value for $E_a$; higher values of $E_a$ would shift the curve to higher temperatures). Note that the curve is flat at very low temperatures (no molecules possess enough activation energy, as shown in the inset), and it plateaus at high temperature (all molecules possess energy equal to, or in excess of the activation energy).

One transformation that frequently encountered from the Arrhenius relation is a plot of the log of $k_{obs}$ as a function of $1/T$. This plot, called an Arrhenius plot, should be linear if the Arrhenius law is followed exactly (and if the parameters *A* and $E_a$ are independent of temperature). The slope of the line is $E_a$ divided by *R*, and the intercept (requiring extrapolation to infinite temperature) is *lnA*. Notice the similarity between this plot and the van't Hoff plot for

[1] Note that this would be a very low value of $E_a$; measurable values of of $E_a$ would shift the curve to higher temperatures, to a more measurable range.

the equilibrium constant[2]. The dependence shown above is plotted below in the Arrhenius format (below). Although the curve shown here is plotted all the way to the *Y*-axis. for real data, only a very narrow band of temperature (probably 20-30K width, usually centered around room temperature, 298 K) would be sampled, and would be centered at 300K. In such a case, extrapolation to the Y-axis (infinite temperature) to determine *A*, is subject to substantial error.




***Collision theory for bimolefular reation—a more mechanistic way to think about Arrhenius.*** The Arrhenius dependence can be rationalized by thinking about the molecular mechanics of the reaction. For a bimolecular gas-phase reaction, three things must happen for a bimolecular reaction to occur. First, reactive molecules must come together. In the gas phase, this is often called a collision, whereas in solution, this is called an "encounter" (more on the difference later). The letter *Z* is often used to describe collision (encounter) frequency, with units of collisions per second per unit volume. Second, the reactive molecules must be in the "right" orientation for reaction to occur in the collision complex. The proportion of molecules in the collision complex with the correct orientation for reaction to proceed, is often represented by a "steric factor", $\rho$. Third, the reactive molecules in the complex must contain enough energy to make, break, or rearrange bonds. As with the Arrhenius equation, we will call this the activation energy $E_a$. The probability that molecules will contain this much (or more) energy is given by the Boltzmann distribution as $e^{(-Ea/RT)}$. Thus, for a bimolecular reaction, the rate constant will be related to these factors as

$$k \propto Z\rho e^{(-E_a/RT)}$$

This expression has a similar form to the Arrhenius law, as long as the *Z* and $\rho$ terms have no (or small, relative to the exponential term) temperature dependences. For a gas-phase reaction, $\rho$ should not depend on temperature. If the collisions are elastic, molecules should spend very little time in the collision complex before flying apart (think billiard balls). Although at higher

[2] This similarity has an historical component as well. Svante Arrhenius' statement of his equation was based on the 1884 work by van't Hoff on the temperature dependence of the equilibrium constant, where van't Hoff noted that his relation implied a similar form for rate constants.

temperatures the reactant molecules should be more rotationally excited, and should be able to change their orientations in shorter time (increasing chances of finding the reactive orientation), the collision time is too short-lived for rotational exploration to make a significant difference. In contrast, the temperature dependence of Z (collision frequency) is non-zero, because as temperatures go up, gas molecules move faster, so there will be shorter time between collisions. The temperature dependence of the average velocity[3]

$$\langle v \rangle = \sqrt{\frac{8kT}{\pi m}}$$

Thus we can expect the collision frequency $Z$ to scale with the square root of temperature. Whether this temperature dependence is seen in comparison with the exponential dependence resulting from the $E_a$ term depends on the magnitude of $E_a$. For large $E_a$'s the exponential term will dominate and the square-root term from $Z$ will not be seen. For $N_2$ gas at STP, a reasonable estimate of Z at 1 atmosphere of pressure is $10^{31}$•$dm^{-3}$•$sec^{-1}$. By dividing by Avogadro's number and multiplying by the molar concentration of gas at STP (1 mol/22.4 liter, or ~0.05 Molar), this is really just accounting for the number of molecules… in a dm3. Simpler to just say it that way.

$$\left(\frac{10^{31}\,collisions}{\sec \bullet\, dm^3}\right)\left(\frac{mol}{6\times10^{23}\,molecules}\right)\left(\frac{dm^3}{0.05 mol\ N_2}\right) = 3\times10^8\,collisions \bullet molecule^{-1}\sec^{-1}$$

it can be seen that each molecule would collide in a very small fraction of a second, and that if all (or even some) gas-phase collisions lead to reactions, these reactions would be over in a nanosecond or so. For most gas-phase reactions, $k_{obs}$ (a bimolecular rate constant times the gas concentration) is much less than this value, suggesting that the reaction is strongly limited by a something else, i.e., a large activation energy.

For unimolecular reactions, it might seem like we could forget about the temperature dependence of $Z$ altogether, because there is no explicit collision in the mechanism. Thus the half-power of $T$ from $Z$ does not enter in. However, unimolecular gas-phase reactions with large values of $E_a$ without collisions are somewhat paradoxical, because there is no obvious way to distribute energy and activate all the reactants. For some resolution to this paradox, see Lindeman's work from 1922.

Note that if there were no activation energy, the reaction would be limited by collision, or encounter, or "diffusion" (which limits the rate of encounter). This is an important consideration, as it gives the maximum reaction rate for a bimolecular process. We will return to this value in more detail later. Also note that by comparing the Arrhenius equation with that derived from collision theory, there appears to be a close analogy between A of the former and $Zp$ of the latter. This will become clearer when we take a thermodynamic approach to thinking about rate constants in the next section.

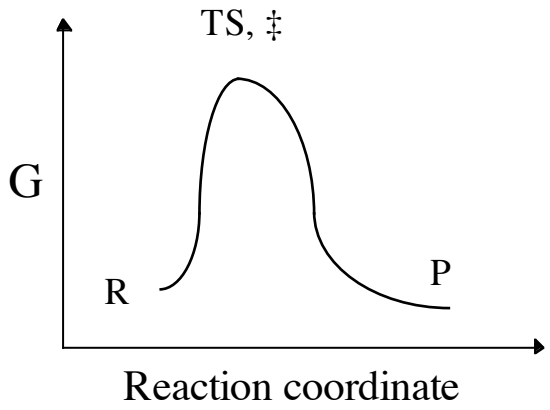
## 2. Transition state theory.

[3] Note this is different from the root-mean-square velocity, by a constant amount. The former is given by

$$v_{rms} = \sqrt{\frac{3kT}{m}}$$

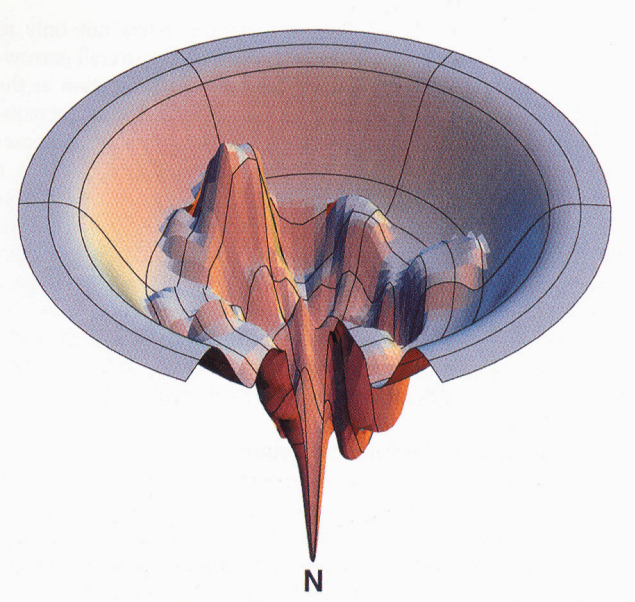
Transition-state theory is a lot like Arrhenius theory, but it develops out of thermodynamic arguments rather than empirical observations of temperature dependence, or considerations of molecular mechanics. It is only applicable with any rigor to single microscopic steps; application to complex reactions involving independent degrees of freedom, nuclear momentum, or multiple steps is questionable, and is likely to corrupt the meaning of extracted parameters from their intended thermodynamic meaning.

In transition state theory, collisions and encounters are not considered explicitly. What is considered is the free energy difference between the ground-state, or reactants, and the highest energy state, called a transition state and represented with a double-dagger (‡) on the **reaction coordinate**. This last pair of words seems to cause a lot of trouble. In all but the simplest cases, the reaction coordinate must be considered as an abstract concept that describes the path taken by a molecule or set of molecules from their reactants to their products. Because we do not have multidimensional paper and multidimensional blackboards, the reaction coordinate is represented abstractly in a single coordinate x-y plot where energy is on y, and reaction coordinate is on x:




However, it should be kept in mind that this is a cut through a multidimensional energy surface. For a simple reaction where the reaction coordinate can actually be described as a single molecular motion (for instance, $S_N2$ hydrolysis of methyl chloride, or cis-trans isomerization of an unsaturated bond), the energy increases in all directions orthogonal to the reaction coordinate. In this regard the TS is a saddle-point, a mountain pass by which two valleys are connected.

A troublesome aspect of the above diagram is that it depicts a "free energy surface", but free energy contains entropy, which is by definition zero for a single conformation. This is not so troublesome for very simple reactions like unimolecular isomerizations, or even for the binding of simple ligands to macromolecules, but for kinetics of major macromolecular conformational transitions, the relevant "species", namely well-ordered, disordered, and transition states, either there are multiple microscopic conformations at each point on the reaction coordinate, or the multidimensionality of the energy surface needs to be depicted. In the former case (multiple conformations at each point on the coordinate) free energy makes sense, because entropy is not always zero, but the "reaction coordinate" looses direct connection with atomic structure. Some attempts have been made recently to increase the dimensionality of the representation, but the representation still depicts only two conformational coordinates and energy.




Such representations are often associated with the term "energy landscape" for obvious reasons. For order-disorder transitions in macromolecules, these representations are still highly oversimplified, but they do highlight the idea that there is more than one way to get from one conformation to another.

Difficulties with depiction aside, transition state theory supposes a transition state conformation (or ensemble of conformations) that is in thermodynamic equilibrium with the reactant. For a unimolecular reaction of the type $A \rightarrow B$, the concentration of the transition state is then given as

$$[\ddagger] = K^{\ddagger}[A] = [A]e^{\left(-\Delta G^{\ddagger} / RT\right)}$$

The rate of reaction is then equal to the concentration of the transition state times the frequency with which the bond breaks, ν:

$$\frac{d[A]}{dt} = -k_1[A] = -\nu[\ddagger] = -[A]\nu e^{\left(-\Delta G^{\ddagger} / RT\right)}$$

Thus

$$k_1 = \nu e^{\left(-\Delta G^{\ddagger} / RT\right)}$$

For a simple bond-breaking reaction, ν can be taken as the vibrational frequency of that bond, which is given by $k_bT/h$, where $k_b$ is Boltzmann's constant, and h is Plank's constant. So defined, ν has a value of ~$6x10^{12}$ $sec^{-1}$. This would be the rate constant for a reaction with no free energy barrier. Thus for this type of reaction,

$$k_1 = \frac{k_b T}{h} e^{\left(-\Delta G^{\ddagger} / RT\right)}$$

This equation is often called the **Eyring equation** after Henry Eyring, an American Physical Chemist and second cousin once removed of Mitt Romney. For a more complicated reaction like an order-disorder transition in a macromolecule, ν cannot be taken as the vibrational frequency of a chemical bond. Some people throw a fudge-factor in front of it all (called κ, kappa) and name it a "transmission coefficient" to take care of reflection back from the transition state,

quantum mechanical tunelling through the barrier, and momentum-type effects. A fudge factor is certainly needed for complicated reactions, unfortunately there is no way to know its value. Although the uncertainty of this factor seems to render transition state theory quite useless for complicated reactions, by comparing rate constants for very similar reactions this unknown factor can be made to cancel (more or less). Consider two reactions which will be distinguished by a prime. Their rate constants $k_1$ and $k'_1$ can be related as follows:

$$\frac{k_1}{k_1'} = e\left(\left\{\Delta G^{\ddagger\prime} - \Delta G^{\ddagger}\right\}/RT\right) \quad \text{or}$$

$$RT\ln\left(\frac{k_1}{k_1'}\right) = \Delta G^{\ddagger\prime} - \Delta G^{\ddagger} = \Delta\Delta G^{\ddagger}$$

Thus by comparing the rate constants of the two processes, differences between energies of the transition states (relative to the reactant(s)) can be inferred for the two processes. An example of where this formalism is used is in comparing the folding rates of wild-type and mutant proteins, where such analysis can be used to guess about which parts of the protein are structured in the transition state. We will look at example of this from the laboratories of Alan Fersht and Bob Matthews in the next lectures.

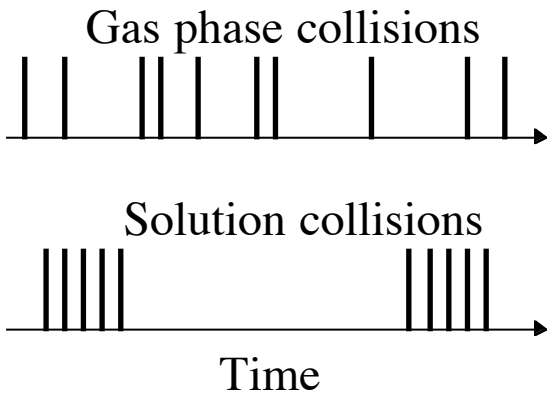
The Eyring equation can be taken further by recognizing that $\Delta G^{\ddagger}=\Delta H^{\ddagger}-T\Delta S^{\ddagger}$. Thus, the equation can be written as

$$k_1 = \frac{k_b T}{h} e^{\left(\Delta S^{\ddagger}/R\right)} e^{\left(-\Delta H/RT\right)}$$

Notice that if the transition state is lower in entropy than the reactant(s), $\Delta S^{\ddagger}$ is negative, and $k_1$ is slowed. This is an entropy bottleneck. For the folding of a complex polymer, if the transition state ensemble contains a small number of conformations compared to the unfolded ensemble, such an entropy bottleneck will occur. As advertised, this equation looks just like the Arrhenius equation. The prefactor A of the Arrhenius equation can be taken as $(k_bT/h)\exp\{-\Delta S^{\ddagger}/R\}$. From collision theory we noticed a close parallel between A and Zp (the product of the collision frequency and the steric factor; see above). It makes sense that Zp would be related to the entropy difference between the reactants and the transition state. A low probability of being in the right orientation means there are many other orientations (which do not react), assuming all orientations are equal. This means the transition state, which requires the correct orientation, has a lower entropy than the reactants, which is exactly the picture from transition state theory.

**3. Bimolecular reactions in solution and diffusion.**

Reactions in solution differ from reactions in the gas phase in terms of how their collisions are distributed as a function of time. Whereas the distribution of collisions of reactant molecules in the gas phase is random (or Markovian, the occurrence of one collision does not affect the likelihood of another), in solution collisions are clustered.




Each set of clusters is often referred to as an encounter. One physical explanation that is given for the clustered nature of collisions in solution is that in order to collide once, two reactant molecules must enter into the same "**solvent cage**". It takes some time for them to get out of this cage (on the order of $10^{-11}$ seconds), and during this time the reactant molecules continue to collide because after each collision they are reflected back by the solvent cage. In essence, during the time of caging, the reactant molecules can be thought of as a gas of two molecules at very high concentration because they are in a very tiny vessel (the cage). In addition, it takes longer to get into the same solvent cage, so encounters in solution are less frequent than collisions in the gas-phase (though collisions can be the same, overall).

Another, less mechanical way of explaining clustering is that movement of molecules in solution is Brownian or diffusional, rather than the "free-flight" motion of a gas, where the only thing that causes gas molecules to change their velocities is collisions with other gas molecules (or walls). Brownian motion is said to sample space very thoroughly at small distances, so the many collisions per encounter are just a manifestation of thorough sampling.

We want to explain a simple bimolecular reaction,

$$A + B \xrightarrow{k_2} P \quad \text{where}$$

$$\frac{\mathrm{d[P]}}{\mathrm{dt}} = k_2[A][B]$$

in a way that captures the diffusive and cage nature of solution reactions. Given the nature of the encounters--reactant monomers alternate between periods of association and dissociation--a biomolecular reaction of A and B (or A and A) can be reasonably modeled as follows:

$$A + B \underset{k_{-d}}{\overset{k_d}{\rightleftharpoons}} AB \xrightarrow{k_1} P$$

The species AB above is the encounter complex, and its rate of formation, governed by $k_d$, will depend on diffusion. In our model, we will not require any activation energy for formation of AB, they must just diffuse together and touch. We partition any necessary activation energy into the chemical step, $k_1$, which can be extracted from the solvent, or perhaps from the molecules themselves if they entered the cage in a “translationally hot state” (i.e. it is moving fast). We will apply the steady-state approximation to the encounter complex:

$$\frac{d[AB]}{dt} = k_d[A][B] - k_{-d}[AB] - k_1[AB] = 0$$

$$[AB](k_{-d} + k_1) = k_d[A][B]$$

$$[AB] = \frac{k_d[A][B]}{(k_{-d} + k_1)}$$

And in this steady state scheme, the velocity of the reaction (expressed in terms of [P]) can be written as

$$\frac{\mathrm{d[P]}}{\mathrm{dt}} = k_1[AB] = \frac{k_1 k_d[A][B]}{(k_{-d} + k_1)}$$

Recognizing that both of the above expressions for d[P]/dt are valid, the overall rate constant $k_2$ can be expressed in terms of rate constants of encounter ($k_d$), of unreactive separation ($k_{-d}$) and of reaction of the encounter complex ($k_1$):

$$k_2 = \frac{k_1 k_d}{k_{-d} + k_1}$$

As before, we can discuss two limits. In one limit, reaction of the encounter complex ($k_1$) is much faster than unreactive separation ($k_{-d}$). In this limit, the apparent rate constant $k_2=k_d$. This is a "**diffusion limited reaction**" or a "diffusion-controlled reaction". In the other limit, reaction of the encounter complex ($k_1$) is much slower than unreactive separation ($k_{-d}$). In this limit, the apparent rate constant $k_2$ becomes $k_1(k_d/k_{-d})=k_1 \bullet K_{encounter}$. This is called an "**activation-limited reaction**", and the overall rate depends on the encounter complex accumulating sufficient energy from the solvent. Such a reaction would be expected to display Arrhenius behavior through $k_1$. Note that as we have modeled things, the overall reaction rate still depends on the rate of encounter, and still has diffusional nature.

How can we calculate $k_d$, the rate of encounter complex formation? We must use some diffusion theory. Imagine a stationary *A* sitting in solvent that contains *B* molecules. We can draw a sphere around A at radius r, and ask "what is the flow (**F**) of B molecules through the sphere"? **Fick's first law** gives the **flux J** as proportional to the concentration gradient:

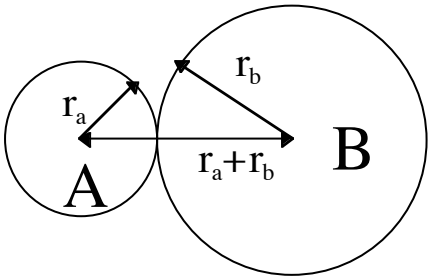
$$J = DN_0 \frac{d[B]}{dr}$$

There is a minus sign that is often in front of the D, depending on how flux is defined. Units of flux are often molecules per second per square centimeter. The square centimeter in the denominator of flux means that it is the amount of stuff that flows through a unit of area. D is the translational diffusion constant, with units that are often $cm^2 \bullet sec^{-1}$. D is a quantity that is particular to the molecule, the solvent, and the temperature. $N_0$ is Avogadro's number, and converts moles to molecules in Fick's law. Note that we had better watch out our units, because [B] has units of $moles \bullet dm^{-3}$, or $10^{-3} moles \bullet cm^{-3}$. The total flow **F** through the sphere of radius r can be given as the flux times the surface area of the sphere:

$$F = 4\pi r^2 D_B N_0 \frac{d[B]}{dr}$$

We use $D_B$ to denote that the diffusion constant is that of B molecules.

For a reaction that is diffusion controlled, when A and B encounter, they react. Our goal is to derive a quantitative expression for the flow at this reactive radius, because this flow sets the rate of reaction. The radius at which A and B react occurs at a radius $r_A+r_B$.




If A and B react at once when they encounter (as expected for a diffusion controlled reaction), the concentration of B and $r_A+r_B$ must be zero. And at r=infinity, the concentration of B is the solution value [B]. We can obtain an expression of the flow at this critical radius by integrating the differential equation above from r=infinity (where the concentration is [B]) to $r_A+r_B$ (where the concentration is zero). Rearranging and integrating,

$$\int_{r_A+r_B}^{\infty} \frac{F}{4\pi r^2 D_B N_0} dr = \int_0^{[B]} d[B]$$

The key to solving this integration comes by recognizing that F is independent of r at values equal to and larger than $r_A+r_B$. This is because no molecules are destroyed until A and B touch. Although the flux increases as r decreases, the surface area of the sphere around A's decrease as r decreases, and these two effects compensate. Thus, most of the terms on the left-hand integral can be taken outside:

$$\frac{F}{4\pi D_B N_0} \int_{r_A+r_B}^{\infty} \frac{1}{r^2} dr = \int_0^{[B]} d[B]$$

$$-\frac{F}{4\pi D_B N_0} \frac{1}{r}\Bigg|_{r_A+r_B}^{\infty} = [B]$$

$$F = [B] 4\pi D_B N_0 (r_A + r_B)$$

The rate of the diffusion-controlled reaction at an A molecule is given by this flow. The total rate of reaction also depends on the concentration (in number of molecules per unit volume, to keep consistent with our expression so far) of A, which is given by $N_0[A]$, so we can write for the total rate $4\pi D_B {N_0}^2 (r_A + r_B)[A][B]$. Thus the constants multiplying [A][B] are nearly equal to $k_d$, the rate constant for formation of the encounter complex, and equal to $k_2$ since we are in the diffusion limit; the equality is exact if we divide by an Avogadro's number so that the resulting expression is in moles per unit volume per second, and not molecules per unit volume per second. Doing so gives

$$k_d = k_2 = 4\pi D_B (r_A + r_B) N_0$$

By allowing A molecules to move too, we effectively increase the diffusion of the system, and accordingly we replace $D_B$ with $D_A+D_B$. By using the Stokes-Einstein relation (for a spherical A and B), we can replace these D's by

$$D_A + D_B = \frac{kT}{6\pi\eta r_A} + \frac{kT}{6\pi\eta r_B} = \frac{kTr_B}{6\pi\eta r_A r_B} + \frac{kTr_A}{6\pi\eta r_B r_A} = \frac{kT(r_A + r_B)}{6\pi\eta r_A r_B}$$

Where η is the viscosity. The viscosity of water is 0.001 kg•m$^{-1}$•sec$^{-1}$ at 20 °C (or in cgs units 0.01 g•cm$^{-1}$•sec$^{-1}$=1 centiPoise). Substituting this into the expression above gives

$$k_d = k_2 = 4\pi(r_A + r_B)^2 N_0 \frac{kT}{6\pi\eta r_A r_B} = \frac{2RT}{3\eta}\frac{(r_A + r_B)^2}{r_A r_B}$$

This is the expression for the diffusion limited rate constant. This is called Smolouchovski equation. Notice that if $r_A=r_B$, it becomes

$$k_d = k_2 = \frac{2RT}{3\eta}\frac{(2r_A)^2}{r_A^2} = \frac{8RT}{3\eta} \quad \text{or}$$

$$k_d = \left(\frac{8}{3}\right)\left(8.314\frac{kg \bullet m^2}{s^2 \bullet mol \bullet K}\right)(298K)\left(\frac{m \bullet s}{10^{-3} kg}\right)$$

$$= 6.6\times10^6 \frac{m^3}{s \bullet mol} = 6.6\times10^9 \frac{dm^3}{s \bullet mol}$$

The last expression is around 7x10$^9$ M$^{-1}$•sec$^{-1}$. Notice that if $r_A$ is not equal to $r_B$, $k_d$ can be significantly increased. For instance, if $r_A$ is $10r_B$ (or vice versa, the theory is symmetric) then

$$\frac{(r_A + r_B)^2}{r_A r_B} = \frac{(11r_B)^2}{10(r_B)^2} = \frac{121}{10} = 12.1$$

Compared with the case where $r_A=r_B$, this difference between $r_A$ and $r_B$ provides about a three-fold enhancement in the diffusion-limited rate constant (remember that for $r_A=r_B$, we obtained the number 4 out of $(r_A+r_B)^2/r_Ar_B$). This is a reasonable scaling for a reaction of a protein like myoglobin with an $O_2$ molecule. Bigger mismatches yield much bigger enhancements in the diffusion-limited rate constant, but it is not likely that reactions involving such big differences in size proceed at the diffusion limit. Since the size of matter is quantized into lumps called atoms, there is a lower limit to how small something can be. $10^{-10}$ m is a reasonable lower limit. Although combination with a molecule with $10^{-8}$ m radius could have an enhanced $k_d$ twenty-five fold (100/4) times faster than $r_A=r_B$, it would require the whole surface of this now big macromolecule to be reactive. It is more likely that there is a small, ~$10^{-10}$ m spot on the big molecule that is capable of reacting, so even for a big molecule and a small one, $r_A=r_B$ is probably a good approximation.

**4. Enzyme kinetics**

It is hard to overstate the importance of enzymes in biology, biotechnology, and industry. Besides central roles in controlling metabolic flux, synthesis of macromolecules, and propagation and expression of genetic information, enzymes are essential for industrial processes

such as detergent manufacture, cheese making, and production of wine, beer, and spirits. Even for molecular biology, genes could not cloned or sequenced without enzymes.

In its simplest form, the reaction we will be talking about is as follows:

$$S \rightarrow P$$

S is substrate and P is product. Where is the enzyme? It is a catalyst. It changes the rate of the reaction, but it does not enter into the stoichiometric equation. This means the enzyme does not get altered by the reaction--it does not get used up. To see the action of the enzyme, we must break the stoichiometric equation down into its mechanistic steps. Then we see the enzyme.

Something I should have mentioned last lecture, one thing that can be obtained from the stoichiometric equation is an expression for the equilibrium constant (assuming the reaction is in equilibrium). For the reaction we discussed yesterday,

$$A + 2B \rightarrow 3C + D,$$

$$K_{eq} = \frac{[C]^3[D]}{[A][B]^2}$$

Thus for the reaction we are discussing here, S->P, the equilibrium constant can be written as

$$K_{eq} = \frac{[P]}{[S]}$$

Again, the enzyme is not there. It is a catalyst, and **catalysts (enzymes) do not change the position of equilibrium**, only the rate at which equilibrium is attained.

There are several differences in the form of the data for enzyme kinetics compared with other types of kinetics, like those of protein folding. First, the enzyme is not consumed in the reaction (see above), except in being transiently bound by substrate. Second, enzymes are hard to get a hold of in large quantities, so enzymatic reactions are usually measured at very low concentrations of enzyme. If substrate was stoichiometric with enzyme at the start of the reaction, the complete conversion would have a tiny amplitude, and the tiny amount of product (or substrate consumed) would be difficult to detect. Thus, a large excess of substrate is present, and the results of many rounds of enzymatic reaction are determined. Since the substrate is at such a high concentration, it does not really change its concentration either. If the concentration of substrate does not change significantly over time, and enzyme is not consumed during the reaction, the velocity of the reaction, which one may expect to be written as some overall rate law

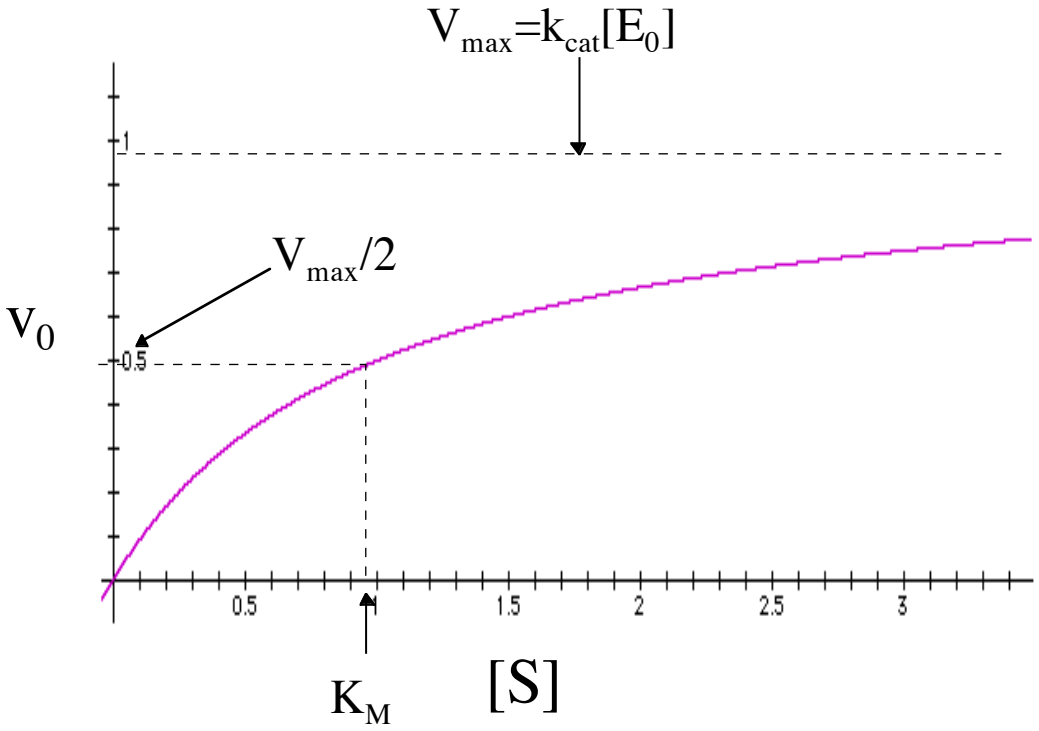
$$V = \frac{d[P]}{dt} = k[E]^n[S]^m,$$

should be independent of time (since all terms on the RHS are independent of time. n and m are unspecified orders, which turn out to change with [S], as will be seen below.). Thus for enzyme kinetics, velocity is very easy to extract from the data: a plot of [P] versus t gives a straight line, whose slope is V at all places (this rate is often called the "initial rate", or $V_0$, in recognition of the fact that at very long times V would decrease as substrate concentration falls). So for enzyme kinetics, it is easy to work with the differential form of the rate law rather than the integrated form, since velocities are so easy to get.

Following the sequence in Fersht's book, I will describe enzyme *steady-state kinetics*, which is used for 95% (my estimate) of studies of enzyme kinetics. First I will describe how $V_0$ varies with substrate concentration, which forms the basis for the Michaelis-Menten equation. It is found that as [E] rises, $V_0$ rises linearly, indicating that n=1 (the order of the reaction in E). It is found that at low concentrations of S, $V_0$ rises nearly linearly with [S], as would be expected in the above equation of m=1 (the order of the reaction in S). However, at high concentrations of S, $V_0$ reaches a limiting value (called $V_{max}$), as if m=0 (zero order in S). Thus it appears that the enzyme becomes saturated with S. Quantitatively this behavior can be well represented by an empirical equation called the Michaelis-Menten equation:

$$V_0 = \frac{V_{\max}[S]}{K_M + [S]} = \frac{k_{cat}[E]_0[S]}{K_M + [S]}$$

This function does the right things at low and high [S]: $v_0$ becomes $V_{max}[S]/K_M$, and $V_{max}$ respectively. The parameter that controls where the transition from one regime to the other occurs is $K_m$ (termed the Michaelis constant), which is equal to the concentration at which $V_0=0.5V_{max}$. This is plotted below:




Michaelis and Menten proposed (in 1913) a kinetic scheme that gives mechanistic meaning to the above parameters. Because their scheme is one limit of the steady-state assumption of a slightly more general mechanism, we will analyze this general mechanism. The reaction scheme we will write, first considered for enzymes by Briggs and Haldane in 1925 is a familiar one:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{k_2} EP \xrightarrow{k_3} E + P$$

For our purposes, we will assume that P dissociates very quickly from E, so that the $k_3$ step is kinetically silent. We apply a steady-state approximation to ES:

$$\frac{d[ES]}{dt} = k_1[E][S] - [ES](k_{-1} + k_2) = 0$$

Since the total enzyme concentration $[E]_0=[E]+[ES]$, we can substitute out the unmeasurable free enzyme concentration by $[E]=[E]_0-[ES]$:

$$k_1([E]_0 - [ES])[S] - [ES](k_{-1} + k_2) = 0$$

$$[ES](k_{-1} + k_2 + k_1[S]) = k_1[E]_0[S]$$

$$[ES] = \frac{k_1[E]_0[S]}{(k_{-1} + k_2 + k_1[S])} = \frac{[E]_0[S]}{[S] + (k_{-1} + k_2)/k_1}$$

Then, recognizing that $V_0= k_2 [ES]$ leads to the expression

$$V_0 = \frac{k_2[E]_0[S]}{[S] + (k_{-1} + k_2)/k_1}$$

This is identical to the Michaelis-Menten equation, where $k_2=k_{cat}$, and $K_M=(k_{-1}+k_2)/k_1$.

**Significance of $K_M$.** In the Briggs-Haldane description, $K_M$ is the ratio of the constants that make ES go away, divided by that which forms it. This it represents the stability of the ES complex. If the rate of transformation of ES to EP is much slower than the rate of dissociation of ES to E+S (i.e. if $k_{-1}>>k_2=k_{cat}$) then $K_M=k_{-1}/k_1$. Thus E and S are in thermodynamic (pre)equilibrium with ES, and the Michaelis constant is a dissociation equilibrium constant for the ES complex. This is often called the "Michaelis complex". Some selected values of $K_M$ for different enzymes are given below.

| Enzyme | Substrate | $K_M$ $M^{-1}$ |
|---|---|---|
| Carbonic anhydrase | $CO_2$ | $8X10^{-3}$ |
| β-Galactosidase | lactose | $4X10^{-3}$ |
| Threonine deaminase | Threonine | $5X10^{-3}$ |
| Pyruvate carboxylase | pyruvate | $4X10^{-4}$ |
| | $HCO_3^-$ | $1X10^{-3}$ |
| | ATP | $6X10^{-5}$ |
| Arginine tRNA synthetase | Arginine | $3X10^{-6}$ |
| | tRNA | $4X10^{-7}$ |
| | ATP | $3X10^{-4}$ |
| Catalase | $H_2O_2$ | 1.1 |

Notice that enzymes with more than one substrate have a $K_M$ for each substrate. $K_M$ typically ranges between $10^{-1}$ and $10^{-6}$, an average exponent is probably $^{-3}$ (i.e. $K_M$=mM).

**Significance of $k_{cat}$.** In cases where product dissociation is fast ($k_3>>k_2$), then $k_{cat}$ is simply $k_2$ as given above. However, if the dissociation of product becomes rate limiting, $k_{cat}$ switches from being equal to $k_2$ to being equal to $k_3$. In other words, if one step is much slower than the next, $k_{cat}$ is equal to the slowest step. When $k_2$ and $k_3$ are near each other in magnitude, $k_{cat}$ is given by $k_2k_3/(k_2+k_3)$. Either way, $k_{cat}$ tells how fast the enzyme can do chemistry when it is bound by

substrate, converting it to free product. $k_{cat}$ is sometimes called the turnover number, because it gives the number of cycles of reaction that an enzyme molecule can catalyze in a second. Some selected values of $k_{cat}$ for different enzymes are given below.

| Enzyme | $k_{cat}$ $sec^{-1}$ |
|---|---|
| Catalase | 40,000,000 |
| Carbonic anhydrase | 600,000 |
| ketosteroid isomerase | 280,000 |
| Acetylcholinesterase | 25,000 |
| Lactate dehydrogenase | 1,000 |
| Chymotrypsin | 100 |
| DNA polymerase I | 15 |
| Lysozyme | 0.5 |

These values of $k_{cat}$ span a large range. Carbonic anhydrase goes very fast: it takes only $1/k_{cat}$=1.7 microseconds to convert ES to E+P. Catalase goes even faster, taking 25 nsec. Other values are slow for good reason. Chymotrypsin and lysozyme both produce covalent intermediates that must be hydrolyzed, and this is a slow step (note that this can be considered slow dissociation of EP through $k_3$, and for these enzymes a covalent bond must be broken for the dissociation). DNA polymerase takes a long time because if it makes a mistake and uses the wrong substrate (dNTP), a mutation results. It spends a lot of time thinking about what it is going to do, and it checks afterwards to make sure it has done the right thing--this is called proofreading, and again it means slow product release (and slow $k_{cat}=k_3$ in this case).

**Significance of $k_{cat}/K_M$: catalytic perfection.** Notice that in the limit of low substrate concentration, the result of the Michaelis-Menten-Briggs-Haldane expression becomes:

$$v_0 \cong \frac{k_2[E]_O[S]}{(k_{-1}+k_2)/k_1} = \frac{k_{cat}}{K_M}[E][S]$$

Substituting [E] for $[E]_0$ is OK because there [S] is much lower than $K_M$, so most of the total enzyme is free. In this limit, $k_{cat}/K_M$ plays the role of an apparent second-order rate constant for the reaction. From our discussion above, we know that this value cannot exceed the diffusion-limited value of around $10^9$ $M^{-1}$ $sec^{-1}$. For some enzymes, $k_{cat}/K_M$ is nearly this high, indicating that in some sense the enzyme is catalyzing its particular reaction almost as fast as it can--if the enzyme could go faster than this, it would clear out all the nearby substrate and have to wait for diffusion to bring it more.

| Enzyme | Substrate | $K_M$ $M^{-1}$ | $k_{cat}$ $sec^{-1}$ | $k_{cat}/K_M$ $M^{-1}$ $sec^{-1}$ |
|---|---|---|---|---|
| Acetylcholinesterase | acetylcholine | $9X10^{-5}$ | $1.4X10^{4}$ | $1.6X10^{8}$ |
| Carbonic anhydrase | $CO_2$ | $1.2X10^{-2}$ | $1X10^{6}$ | $8.3X10^{7}$ |
| Catalase | $H_2O_2$ | 1.1 | $4X10^{7}$ | $4X10^{7}$ |
| Triosphosphate isomerase | glyceraldehyde -3-phosphate | $4.7X10^{-7}$ | $4.3X10^{3}$ | $2.4X10^{8}$ |