LECTURE 1 & 2-Chemical kinetics

Enzymes are proteins that are specialized to catalyze biological reactions. Catalysts are entities that participate in, but are not components of the reactions. That is, CATALYSTS ARE NOT USED UP IN THE REACTION.

Enzymes are characterized by their extremely high degree of *substrate* specificity (SUBSTRATE=REACTANT IN A REACTION) and their amazingly high catalytic power. Some enzymes accelerate the rate of their reactions by ~10^6 over that observed in solution.

Enzymes catalyze (facilitate) the same type of reactions as occur in solution-only they make them occur faster! This high degree of rate acceleration is obtained by bringing the substrates close together (PROXIMITY) and in the proper ORIENTATION. This is effected by the fact that enzymes bind there substrates, there is an intermediate complex formed between E and S, before catalysis. This discussion suggests that ENZYME CATALYZED REACTIONS OCCUR IN MULTIPLE STEPS.

YOU HAVE TO REALIZE-ENZYMES DO NOT ALTER THE EQUILIBRIUM OF THE REACTION!!! THEY CATALYZE ONLY REACTIONS THAT OCCUR SPONTANEOUSLY!!! Some enzymes utilize tricks, coupling favorable and unfavorable reactions to give the desired results, but the free energy of an enzyme catalyzed reaction is always negative.

<u>Kinetics</u>- determination and study of the velocity of chemical reactions. Some reactions are very slow others very fast. In order for a chemical reaction to occur, the reactants must collide with each other and with enough energy to result in a reaction. Any condition that creates a greater number of collisions with the minimum activation energy required will result in a faster reaction.

The speed of a chemical reaction is dependent on all of the following:

1. The temperature at which the reaction occurs

An increase in temperature will result in an increase in kinetic energy. Since the kinetic energy increases, the velocity of the particles will also increase. Since the speed of the particles increases, they should collide more often (therefore speed of reaction increases). The particles will also have more energy thereby speeding up the reaction even more.

2. The concentration of reactants

The more reactant particles there are per unit volume (concentration of reactants) the more chances there are for collisions. This should result in an increase in the speed of the reaction.

3. The presence of a catalyst

A catalyst will speed up a reaction by providing a better geometric arrangement for reactivity (reaction sites line up better) or by providing an alternate pathway for the reaction (one with a lower reaction energy). Although a catalyst may take an active part in the reaction, it can be recovered at the end of the reaction chemically unchanged

4. The nature of the reactants

The nature of the reactants will also affect the speed of reaction. Since reacting particles must come close to each other in order to react, oppositely charged ions would be expected to react very quickly. Large molecules may be slow to react if the reaction site on each reactant is not aligned correctly.

The purpose of the study of enzyme kinetics is to deduce the reaction mechanism. The reaction mechanism determination involves identifying the intermediate steps by which the reactants are converted into products. Having established the reaction mechanism, we examine the effect of the environment on the rate of intermediate formation and breakdown. This probing gives insight into the chemical and thermodynamic mechanisms of catalysis

We will begin with an exploration of the thermodynamic principles that determine the rate constants and finally then a discussion of how catalysts (enzymes) affect the rates of reactions. This will be followed by a discussion of how the experimental determination gives of the # of steps and reactants involved in the reactions.

Kinetic Energy and Chemical Reactions

Consider the conversion of 1 base pair of double-stranded DNA to the unpaired or single-stranded state:

$$\text{dsDNA} \rightleftarrows \text{ssDNA}$$
$$A \rightleftarrows B$$

In order to achieve this transition requires the input of energy. Where does the energy come from?

Solvent!

Solvent molecules transfer their energy to the DNA. The energy inherent in a solvent molecule at any temperature is its kinetic energy. The kinetic energy within a water molecule is equal to:

$$k_e = \tfrac{1}{2} mv^2 \qquad \text{(Eqn. 1)}$$

where v^2 equals the average squared velocity and m equals the mass. To describe the distribution of kinetic energies in a large collection of atoms or molecules, we must resort to statistics. Maxwell and Boltzmann discovered that this distribution may be described by plotting the fraction of molecules in a container with a given kinetic energy vs kinetic energy. Alternately, we can plot the probability that water molecules will have a given kinetic energy vs. kinetic energy

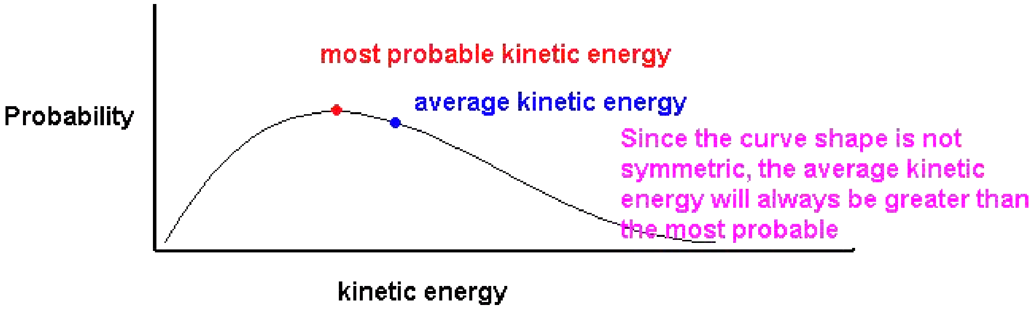


Figure 1

The reason for this odd distribution is that for a collection of particles, for example solution of H_2O molecules, each has a set of energy states available to it. The energy states are quantized states accessible to any particle. If a collection has the same energy, the overall distribution of particles (molecules) is the one that is most degenerate, i.e., the largest number of ways of arranging the particles over the states, is the most likely distribution.

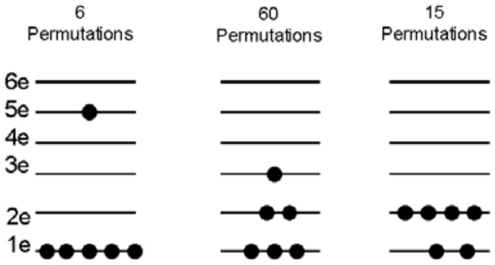


Figure 2

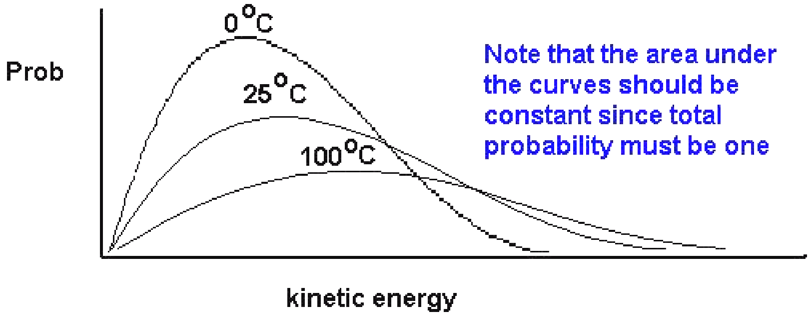
Note that the average kinetic energy of the favored distribution is 1.67e, but the most probable kinetic energy is 1e. This distribution of energies is known at the Boltzmann distribution.

As we all know, kinetic energy is the energy of motion, and as described in Eq. 1, k_e is directly proportional to the square of the speed of how fast the molecules are moving. One way to get things moving faster is to heat them, i.e., change the temperature. In fact the temperature of a solution is directly proportional to the average kinetic energy of molecules. Thus:

$$k_e = 3/2\ nRT \qquad \text{(Eqn. 2)}$$

where n is the number of moles, T is the temperature in Kelvin, and R is the gas constant usually expressed in units of 1.98 cal/deg mole.

If T is proportional to the average kinetic energy, then we'd expect for this change in the average kinetic energy to be reflected in changes in the shape of the Boltzmann distribution. Since in our example we're using probability on the vertical axis, the area under the curve must be exactly equal to 1.0 at all times (there must a unit probability of the water having some kinetic energy). As temperature increases, the curve will spread to the right and the value of the most probable kinetic energy will decrease. This is illustrated in Figure 3 for several temperatures.



Note that the area under the curves should be constant since total probability must be one

Figure 3

It is often useful to fix our attention on some value of the kinetic energy and ask what happens to the fraction of molecules with kinetic energies equal to or greater than this value as we change the temperature. Let's reexamine the curves from above with this in mind. These curves are illustrated below. I have selected a reference kinetic energy in Red.

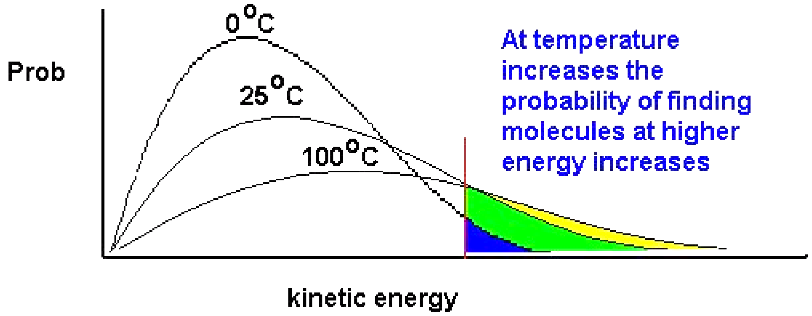


Figure 4

Notice that as T increases, the fraction of molecules with energies greater than the red line increase. This fact is of fundamental importance to properties of matter such as vapor pressures, and we will see that these ideas underlie the effect of temperature on the rates of chemical processes.

To begin evaluating the effect of temperature on a particular process, it is useful to think about the effect of temperature on the kinetic energy of a particular molecule. This is done by scaling Eqn 2 to a *per* molecule:

$$\text{Molecular } k_e = 3/2\ k_B T \qquad \text{(Eqn. 3)}$$

where k_B is the Boltzmann constant. This constant is version of R, the gas constant, scaled on a per molecule basis. Thus:

$$R = N\ k_B = 6.02 \text{ X } 10^{23} * k_B$$

where N is Avogadro's number. Thus, we can rewrite Eqn. 2 for 1 mole of molecules

$$\text{Molar } k_e = 3/2\ NRT \qquad \text{(Eqn. 4)}$$

for any molecule at room temperature (298 K), the average (kinetic) energy a molecule has is 0.89 kcal/mol. This is the amount of energy any one mole of solution has available for transfer. According to the first law of thermodynamics, simply stated as the conservation of energy, each molecule may have different energies, but the total energy, regardless of distribution, must be 0.89 kcal/mol. Rexamining Figures 1-3 and considering our goal of finding out where the energy for breaking a base pair H-bond comes from, we can see that it derives from the transfer of energy from water molecules that have higher k_e that the energy of the base pair bond.

This example, while simplistic, illustrates the basic thermodynamic concept for energy transfer in chemical reactions. Thus, whenever a question arises about the effect of temperature on some chemical process, you should refer back to the Maxwell-Boltzmann concepts.

Evaluation of the Thermodynamics of Activation Parameters
Transition State Theory.

As stated above, before molecules can react, they must attain a particular energetic state, allowing the enthalpic processes to occur, i.e., bond breaking and formation and rearrangement and/or collide with each other, as in a second order reaction, and in doing so, overcome unfavorable entropic factors, orientation and proximity considerations. Thus, there is an energy barrier between reactants and products. That this is true is illustrated by the fact that complex material forms exist, we are not all decomposed, there is some barrier to decomposition. We can illustrate this on an energy diagram, with the energy barrier separating reactants and products called the activation energy.

For the reaction A→B

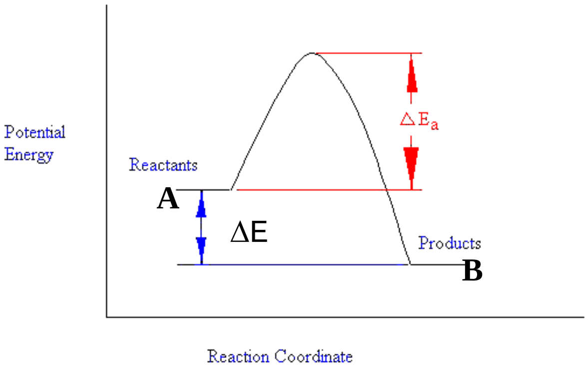


Figure 5

Note that:

- ΔE describes energy difference equilibrium between reactant (A) and product (B). Since the second law of thermodynamics predicts that the energy of the system at equilibrium, the reaction always flows down hill ($-\Delta E$), i.e., the energy of the products is lower than the energy of the reactant

- ΔE_a describes the height of the barrier between the reactant and the transition state.

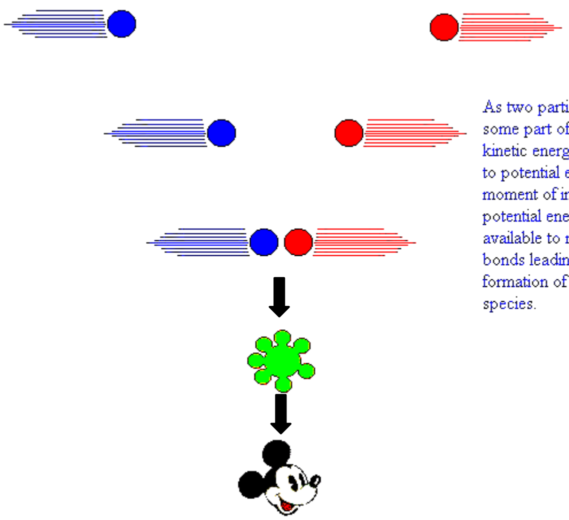
Since the **rate constant of a given reaction actually describes the number of events in a given amount of time** (products formed or reactants consumed), the rate constant is inversely proportional to the height of the activation barrier. To more easily overcome this barrier, the reactants must be *"energized"*.

If an intermediate is much higher in energy than either the reactants or intermediates, where does this energy come from? In line with our discussion of the Boltzmann distribution, the easiest way to energize reactants is to change the temperature, or increase the kinetic energy of the system.

Kinetic Energy and Potential Energy

Let's think of a simple situation in which a car is speeding toward a wall. The car has some kinetic energy that is equal to one half of its mass times its velocity squared. When the car hits the wall, its velocity goes to zero, where does its kinetic energy go to? Obviously it goes into the energy necessary to bend, break and otherwise rearrange the bonds in the frame of the car and the passengers. Modern automobiles have specially designed components that are designed to absorb as much of this energy as possible so that it doesn't go into breaking up the passengers. In summary, the kinetic energy is converted into potential energy at the moment of impact, and this potential energy is available for bond making and breaking. The situation with two molecules is the same.

Let's simplify things and assume that they collide head-on with exactly the same kinetic energy. At the moment of collision, all of the kinetic energy will be converted to potential energy, and this potential energy can be used to make and break bonds leading to a new product. We can illustrate this process in Figure 6.



Source of energy of activation

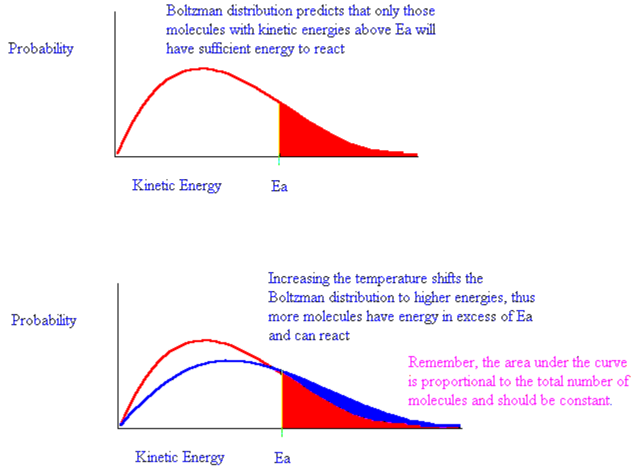
As two particles approach, some part of their total kinetic energy is converted to potential energy at the moment of impact. This potential energy is then available to rearrange bonds leading to the formation of a new species.

Figure 6

In a real collision, some of the energy may remain in kinetic energy, and some of the energy may go into molecular vibrations that don't result in bond breaking and making, thus not every collision necessarily leads to product formation.

If E_a is the minimum energy necessary to get over the energy hill, then we can go back to the Boltzman curve and imagine that only those molecules with energy in excess of E_a will make it over the hill. Figure 7

Effect of Temperature on Reaction Rates - Boltzman Explanation



Boltzman distribution predicts that only those molecules with kinetic energies above Ea will have sufficient energy to react

Increasing the temperature shifts the Boltzman distribution to higher energies, thus more molecules have energy in excess of Ea and can react

Remember, the area under the curve is proportional to the total number of molecules and should be constant.

As shown in Figure 7, an increase in temperature will increase the number of molecules with energies in excess of E_a and hence increase the rate of the reaction.

ArrheniusEquation:

In 1889, Arrhenius suggested the simple relationship between the specific rate constant for a reaction and the temperature of the system.

$$k = Qe^{-E_a/RT}$$

the constant *Q* is a factor that describes the probability that molecules that have sufficient energy to jump the E_a barrier will react, and R is the gas constant and T is the absolute temperature. The more useful form of the Arrhenius equation is given as:

$$\ln k = -E_a/RT + \ln Q$$

Thus if the rate constant of a reaction is measured at several different temperatures and is plotted vs 1/T, a straight line should result, the slope of which is E_a/R.

Arrhenius theorized correctly that the temperature dependence of a reaction must indicate that before products can be formed, the reactants must be in an *activated complex* or as we also call it, *transition state*.

Eyring Theory and Eyring Equation.

Strictly speaking, the Arrhenius equation works only for gas phase, purely *in*elastic collisions. For mixed phase reactions or those that involve elastic collisions, Eyring's theory and equations are used. The basis of Eyring's theory stems from a thermodynamic understanding of equilibrium (Gibb's) processes. Using Eyring's theory, therefore, we can use the concepts of transition state theory to describe the velocity of a reaction in thermodynamic terms.

In the transition state, chemical bonds are in the process of being made or broken. A simple way then of understanding the rate constant for a reaction is to realize that the *transition* state and the *ground state* (reactants) are in thermodynamic equilibrium:

$$A \underset{k-1}{\overset{k1}{\rightleftarrows}} A^*$$

so that the concentration of the transition state is calculated from their difference in energies, the overall rate is then the concentration of the transition state multiplied by the velocity of its productive decomposition.

$$A \underset{k-1}{\overset{k1}{\rightleftarrows}} A^* \;{}^{k_\ddagger}\!\longrightarrow P$$

$$k=K^\ddagger \cdot k^\ddagger$$

We will treat the two terms of this equation separately. For K^+, the equilibrium constant, as we know from standard equilibrium thermodynamics is

$$\Delta G^\ddagger = -RT \ln K^\ddagger,$$

solving for K and taking the antilog:

$$K^\ddagger = e^{-(\Delta G\ddagger/RT)}.$$

The frequency at which a transition state decomposes is the same as the vibrational frequency of the bond which is breaking. According to physical principles, this frequency ν, is equal to E/h, where h is Planck's constant, and E is the average vibrational energy of a bond.Thus:

$$k^\ddagger = \nu=E/h$$

At temperature T, the vibration of an excited bond in a transition state, according to classical physics and our discussions of kinetic energy above, has the value of k_BT, where k_B is Boltzmann's constant (which relates mean kinetic energy to absolute temperature). Since

$$E = k_BT,$$

then

$$k^{\ddagger} = k_BT/h$$

Thus, putting this all together gives:

$$K^{\ddagger} = k_BT/h$$

Therefore, the rate constant, $k=K^{\ddagger}*k^{\ddagger}$ can be expressed in classical physics as:

$$k = k_BT/h\ e^{-(\Delta G^{\ddagger}/RT)}$$

or taking the ln and rearranging:

$$\Delta G^{\ddagger} = -RT * \ln (k * h/k_BT)$$

Since the free energy of a the formation of a transition state can be described by equilibrium thermodynamics equations:

$$\Delta G^{\ddagger} = \Delta H^{\ddagger} - T\Delta S^{\ddagger}$$

Thus, the reaction rate constant can be given as:

$$k = k_BT/h * e^{(-\Delta H^{\ddagger}/RT)} * e^{(\Delta S^{\ddagger}/R)}$$

or taking the ln:

$$\ln (k/T) = -\Delta H^{\ddagger}/RT + \Delta S^{\ddagger}/R + \ln k_B/h$$

$$y \qquad m \quad x + \qquad b$$

(the ln k_B/h term is constant & small over a small range of T)

Thus a plot of ln k vs 1/T allows evaluation of the $\Delta H^{\ddagger}$ from the slope and $\Delta S^{\ddagger}$ from the intercept

Significance and application of transition state theory

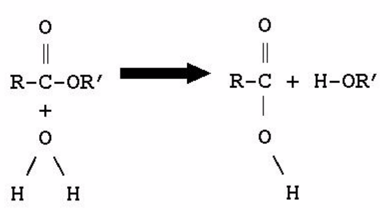
The significance of the transition state theory lies in the understanding of the factors that affect equilibrium constant between the activated complex and the ground state. The rate constant for a reaction is directly proportional to this term; i.e., the slower the reaction, smaller the equilibrium constant, indicating a high energy barrier (large positive $\Delta G^{\ddagger}$). By assessing various factors, e.g., structure of the substrate or solvent conditions, that affect the rate constant for a particular reaction, we can get insights into the reaction's chemical mechanism.

A useful guide in the application of transition state theory to understanding solution effects or structure-reactivity data on rate processes is the **Hammond Postulate.** This states that if there is an unstable intermediate along the reaction pathway, then the transition state will resemble this intermediate. This is a useful way to guess the structure of the transition state and thereby predict the type of stabilization it will require. For understanding enzymic reactions, this is of critical importance for two reasons:

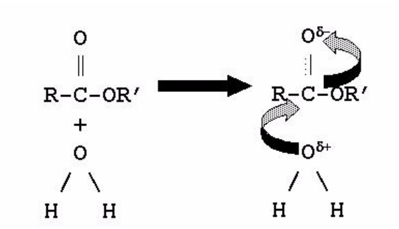
1) enzymes accelerate reactions by lowering the energy of the transition state. That is, they decrease $\Delta G^{\ddagger}$. An insight into transition state structure tells the reaction mechanism.

2) the best enzyme inhibitors are often those that mimic the transition state, because they are so specific and bind very tightly. This then points out one of the uses of enzyme studies-development of new enzyme inhibitors which may be able to function as drugs or biochemical tools.

To understand the transition state and how catalysts may increase the rate by stabilizing this state, let us consider the hydrolysis of an ester:

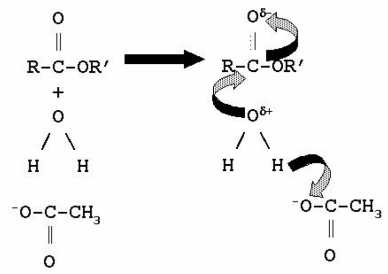


The uncatalyzed attack by water on an ester (as present in proteins) leads to a transition state which has a carboxonium ion as an intermediate:



The formation of this transition state is very unfavorable because of the unstable charges that are developed. Clearly, the transition state would be stabilized if the partial negative charge on the carboxyl oxygen were stabilized by a positively-charged proton donor and if one proton of the attacking water were drawn off by a proton acceptor.

A partial solution to transition state stabilization <u>in solution</u> can be obtained by performing the hydrolysis reaction in the presence of acetate.



This type of catalysis is known as *general base catalysis*, since one of the protons from water is transferred to a base during the reaction.

Entropy and the role of intramolecular catalysis So this is how reactions in solution are catalyzed-stabilization of transition states. But enzyme catalyzed reactions are characterized not only by this process, but also by their incredible efficiency. The question at issue then becomes, why are enzymes such efficient catalysts? The answer lies in the principle of *effective concentration*. In a solution, the catalyst, which in our example above is the acetate molecule, must find the substrate (the ester) and the water, and all must form a complex having the proper orientation of the reactants. Enzymes on the other hand contain the catalysts (for the ester hydrolysis reaction, the general acid and base catalysts) <u>present in one molecule</u>-and by virtue of its highly defined tertiary structure, when the substrate binds to the enzyme, they are <u>in the proper orientation</u> for reaction. These <u>proximity and orientation effects</u> can combine to increase the effective concentration of a catalyst to 13M, in esterases.

This binding of substrate and catalysts into a tightly bound complex alters the reaction which forms the transition state from one which is wholly **intermolecular** to one which is **intramolecular**. This change from **inter-** to **intramolecular** leads to a rate acceleration due to changes in the entropy of the transition state. In a solution reaction, the water, substrate and catalyst must come together in proper orientation, leading to a huge decrease in the entropy of the system-rendering the $\Delta G^{\ddagger}$ huge, thereby slowing the reaction tremendously. In an enzyme reaction however, the bound substrate and catalytic groups are already properly oriented, thus there are little, if any, changes in entropy during the catalytic reaction, its rate then is mostly dominated by enthalpy considerations of bond breaking or making.

Of course most of you are saying, 'but there is an entropy decrease upon binding substrate, how is that paid for?' First, you must realize that the entropy of substrate binding occurs in previous, thermodynamically distinct, separate step. The '*entropy cost*' is paid for by complimentary interactions between groups on the enzyme and substrate-i.e., a number of interactions with favorable ΔH pay for the substrate binding entropy.

Therefore, enzymes are poised to be effective catalysts for two reasons:

1) they pay for substrate entropy loss with favorable enthalpic interactions in a binding step.
2) they eliminate any unfavorable entropy effects in the formation of a transition state by having the catalytic groups positioned in a favorable <u>orientation and in close proximity.</u>