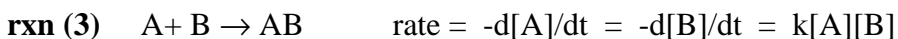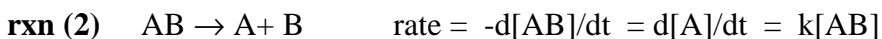# Review of Chemical Kinetics   7.51 September 2001

Kinetic experiments study the rate at which reactions occur, that is how the concentration of some molecular species changes as a function of time. In a plot of concentration vs. time, the **rate of the reaction** is simply the slope. In the kinetic experiment shown below, the rate of the reaction decreases as the reaction proceeds.
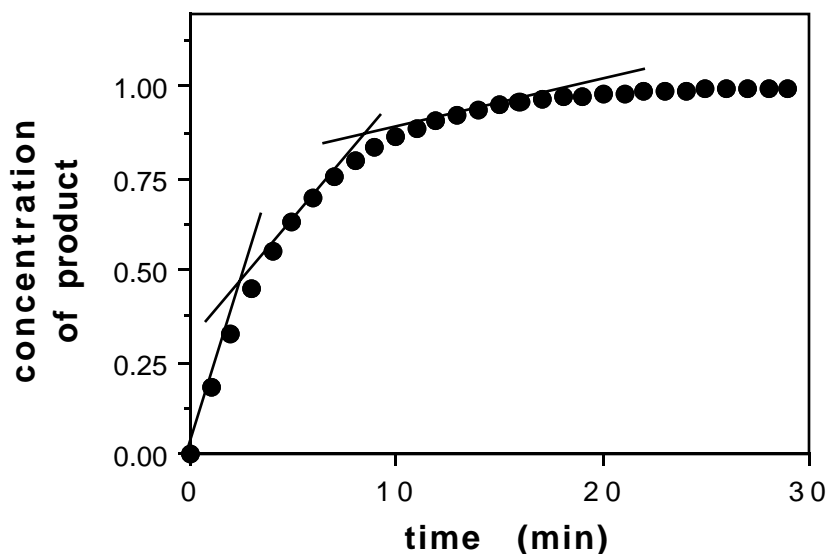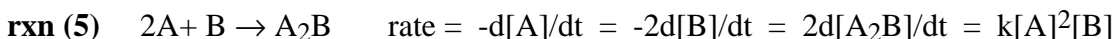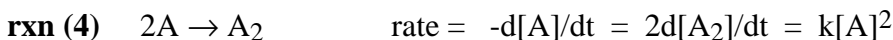

The exact way in which reaction rates change as a function of time and how these rates vary with the concentration of reactants depends on the reaction mechanism. Kinetic studies can be used, therefore, to test proposed mechanisms.

Rate equations or **rate laws** show the change in the concentration of one molecular species with respect to time (the **rate**) as a mathematical function of a **rate constant** or **kinetic constant,** specified by a lower case k, and the concentrations of each molecular species that participates in the reaction.

The examples shown below are rate laws for reactions that proceed in a single direction (i.e., back reactions are not considered). In some of the cases shown, certain rates have been adjusted to be consistent with the stoichiometry of the reaction. Thus, for $2A \rightarrow A_2$, the monomer concentration changes twice as fast as the dimer concentration.

rxn (1) $U \rightarrow N$ rate $= -d[U]/dt = d[N]/dt = k[U]$

rxn (2) $AB \rightarrow A + B$ rate $= -d[AB]/dt = d[A]/dt = k[AB]$

rxn (3) $A + B \rightarrow AB$ rate $= -d[A]/dt = -d[B]/dt = k[A][B]$

rxn (4) $2A \rightarrow A_2$ rate $=$ $-d[A]/dt$ $=$ $2d[A_2]/dt$ $=$ $k[A]^2$

rxn (5) $2A + B \rightarrow A_2B$ rate $=$ $-d[A]/dt$ $=$ $-2d[B]/dt$ $=$ $2d[A_2B]/dt$ $=$ $k[A]^2[B]$

Note that in each of the reactions shown above, the reactants and products are different molecular species. This means that all of the reactants have participated in the reaction. This is not true, however, for the following reaction.

rxn (6) $A + B + C \rightarrow AB + C$ rate $= -d[A]/dt$ $=$ $-[B]/dt$ $= k[A][B]$

Here, the rate law is identical to rxn (3) and the concentration of [C] doesn't appear in the rate expression because C does not participate in this reaction.

Rates are expressed in units of concentration/time (for example, $M \cdot sec^{-1}$, $\mu M \cdot min^{-1}$, $nM \cdot sec^{-1}$, etc.). The units of the rate constant always contain reciprocal time (sec^{-1}, min^{-1}, etc.) and depending on the reaction may also contain one or more reciprocal concentrations. For example, if the right side of the rate equation is $k[A][B]$, then the rate constant must have units of $M^{-1} \cdot time^{-1}$ so that the entire expression will have units of $M \cdot time^{-1}$.

Order of reaction. The overall kinetic order of a reaction is defined by how many concentrations appear on the right side of the rate expression. The order of the reaction with respect to a particular species is defined by whether that species appears one or more times. For example, if the right side of the rate law is $[A]^m[B]^n$, then the overall order of the reaction is m+n and the reaction is m'th order with respect to [A] and n'th order with respect to [B]. Zeroth order means that the reaction rate does not change as the concentration of a species is changed. There are no biochemical reactions that are zeroth order overall.

For the reactions shown above, rxn (1) and rxn (2) are first-order reactions because just one concentration appears on the right side of the rate expression). Rxn (3) is second order overall, first order with respect to [A], and first order with respect to [B]. Rxn (4) is second-order overall, and second order with respect to [A]. Rxn (5) is third order overall, second order with respect to [A]. and first order with respect to [B]. Reaction (6) is is second order overall, first order with respect to [A], first order with respect to [B], and zeroth order with respect to [C].

Units and magnitude of rate constants. For first-order processes, including conformational changes (rxn 1) and dissociation reactions (rxn 2), the rate constant has units of time^{-1}. For second-order or bimolecular association processes, like rxn (3) and rnx (4), the second-order rate constant has units of M^{-1} time^{-1}.

There are upper limits on the magnitudes of first-order and second-order rate constants. For first-order processes, the fastest reactions can't exceed the rates of molecular vibrations or bond-rotations and thus there is an upper limit of approximately 10^{12} sec^{-1} for rate constants. The fastest protein folding reactions known occur with rate constants of about 10^6 sec^{-1}. Simple conformational changes may occur with rate constants of 10^9 sec^{-1}. For second-order reactions, the upper limit on rate constants is imposed by the rate of diffusion, approximately 10^9 M^{-1} sec^{-1}, since two molecules can't react if they don't collide. There are no lower limits on rate constants. In general, for a given set of concentrations, large rate constants mean fast reactions and small rate constants mean slow reactions. Note, however, that a large rate constant does not ensure a large rate. The bimolecular reaction $A+B \rightarrow AB$ may have a diffusion-limited rate constant (10^9 M^{-1} sec^{-1}) but will not occur at any rate if the concentration of [A] or [B] is zero.

Reaction coordinate diagrams, like the one shown below for a protein denaturation reaction, are often used to illustrate the idea that there are energy barriers to kinetic processes. The highest, least stable point on the free energy landscape is called the **transition state**. Even if a reaction is energetically favored (it proceeds to a lower free energy), there is a certain amount of activation free energy that is required to get the reaction to occur. For a protein unfolding reaction, $\Delta G_u^{\ddagger}$ is largely the energy required to break the noncovalent bonds that hold various parts of the structure together. In reaction coordinate diagrams, large barriers (large values of $\Delta G^{\ddagger}$) correspond to slow reactions and vice versa.
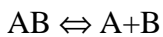

$$N \longrightarrow U$$
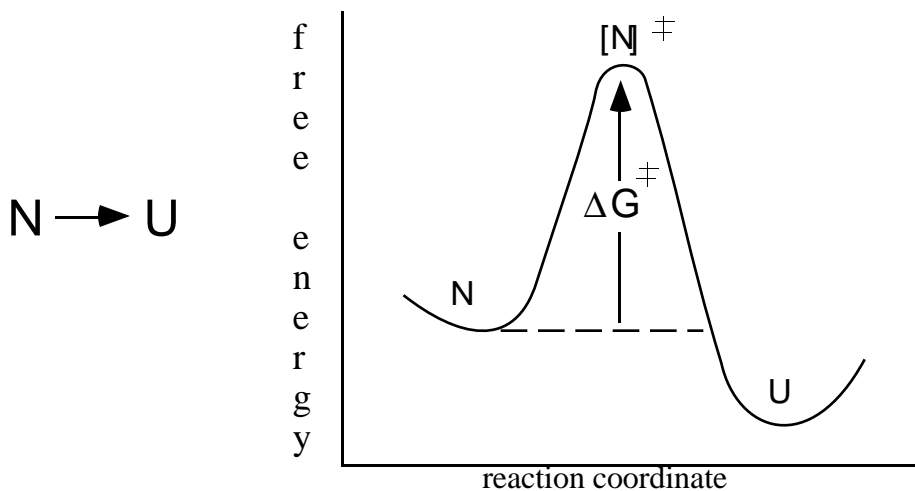
Rate expressions for dynamic systems.   Relationship of kinetic and equilibrium constants.

There are relatively few reactions in biology that are truly irreversible. Thus, when we write a reaction like:

$$AB \rightarrow A+B$$
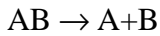
it may not be reasonable or possible to ignore the reverse reaction. If we consider both the dissociation and association reactions together,

$$AB \Leftrightarrow A+B$$

then our combined rate law is:

$$d[AB]/dt = k_{on}[A][B] - k_{off}[AB]$$

where k_{on} and k_{off} signify the association and dissociation rate constants, respectively. The $k_{on}[A][B]$ portion is the rate at which AB is made from the association of A+B. The $-k_{off}[AB]$ portion is the rate at which AB is lost by dissociation. When the system comes to equilibrium, there will be no net change in the concentrations of [AB], [A], and [B]. Thus,

$$d[AB]/dt = k_{on}[A][B] - k_{off}[AB] = 0 \quad \textbf{and} \quad [A][B]/[AB] = k_{off}/k_{on} = K_d$$

This shows the relationship between the rate constants and the equilibrium constant for a bimolecular dissociation reaction. For a unimolecular protein unfolding/folding reaction at equilibrium.

$$N \Leftrightarrow U \quad d[N]/dt = k_f[U] - k_u[N] = 0 \quad \textbf{and} \quad [U]/[N] = k_u/k_f = K_u$$

Measuring rates and determining rate constants: There are several main issues in designing experiments to measure rate constants. First, one must have an assay that can distinguish between reactants and products. This could involve spectral assays (UV absorbance, fluorescence, circular dichroism, NMR, plasmon surface resonance, etc.) or assays in which reactants and products are separated and identified by their mobilities on gel filtration, HPLC, electrophoresis, etc.

Second, the experiment must be set up so that a single process (for example, dissociation or association) predominates. For a dissociation experiment, one might start with AB complexes and then dilute to a sufficiently low concentration so that once complexes fall apart the rate of complex reassociation would be negligible. For an association reaction, A

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and B could be mixed together at time zero and time points could be taken in a regime where no substantial dissociation of AB complexes would be expected (e.g., before much AB is formed) or under conditions where the rate of AB dissociation is negligible. To measure the rate of a unimolecular process like protein folding, one might start with denatured protein at low pH or in a denaturant like urea and then jump into buffer at pH 7 where folding is favored. As any reaction approaches equilibrium, the forward and reverse rates become similar. In general, then, to study the kinetics of the forward or reverse reactions in isolation, one works as far from equilibrium conditions as possible.

The final issue is how to analyze the kinetic data to determine rate constants. The examples below cover simple ways to analyze the kinetics of unimolecular or bimolecular reactions.

First order (unimolecular) reaction

examples: conformational change or dissociation reactions

Reaction: $$A \rightarrow B$$

Rate Law: $$-d[A]/dt = k[A]$$

Integrated solution:

$$\int d[A]/[A] = -k \int dt$$

$$\ln [A] = -kt + \ln [A_o]$$

$$[A] = [A_o] e^{-kt}$$

For any first-order reaction (like $A \rightarrow B$), there will be an **exponential** decay in the concentration of reactant, as shown in the left panel of the plot below, for a reaction with $[A_0] = 10^{-3}$ M and $k = 0.1$ sec^{-1}. Because product is made as reactant is lost, there would also be a corresponding exponential increase in the concentration of product.

For any first-order reaction, there is also a linear relationship between ln [reactant] and time as shown in the right panel. The slope of this semi-log plot is simply -k. Note, however, that plotting ln [product] vs. time does not give a straight line.

exponential kinetics

exponential kinetics
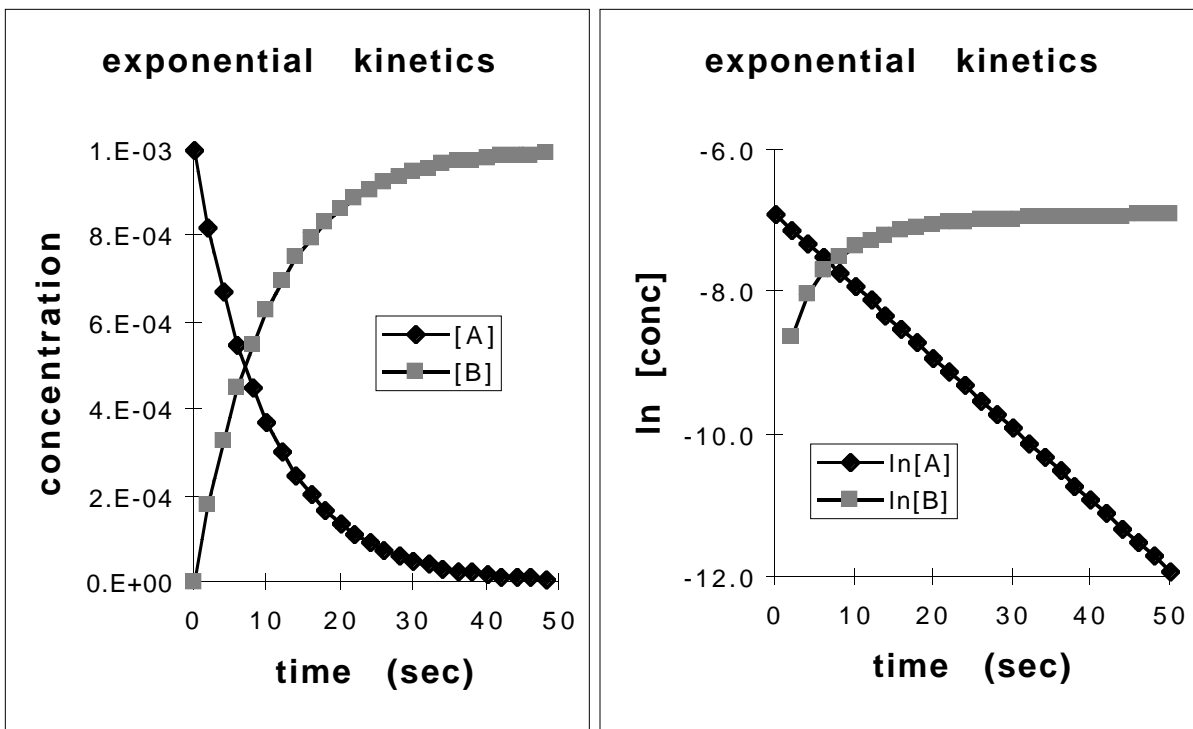
A good linear fit to experimental kinetic data plotted as ln [reactant] vs. time provides evidence that a reaction is first order or pseudo first order (see below).

The **half-life** ($t_{1/2}$) for a reaction is the time required for half of the reactants to convert to products. For a first-order reaction, $t_{1/2}$ is a constant and can be calculated from the rate constant as:

$$t_{1/2} = -\ln(0.5)/k = 0.693/k$$

In the experiment shown above, the half-life is 6.9 sec. This reciprocal relationship between half-life and the rate constant is a useful way for getting a sense of how long a given reaction will take. Thus, for $k = 0.01$ sec^{-1}, the half-life would be about 70 sec. For $k = 10$ sec^{-1}, the half-life would be about 0.07 sec or 70 milliseconds. The half-life, for first-order reactions, is also independent of the starting point. If it takes 20 sec for the first half of the molecules to react, it will also take 20 sec for half of the remaining molecules to react, and so on. The fact that the half-life is constant during a unimolecular reaction means that, at any point in time, a constant fraction of the reactant molecules have sufficient energy to cross the kinetic barrier to become product molecule. This makes sense because the energy in a population of molecules is apportioned randomly according to a Boltzmann distribution.

Second-order reaction (dimerization)

Reaction: $$2A \rightarrow A_2$$

Rate Law: $$-d[A]/dt = k[A]^2$$

Integrated solution:

$$\int d[A]/[A]^2 \; = \; -k \int dt$$

$$1/[A] \; = \; k\,(t) + 1/[A_0]$$

$$[A] \; = \; A_0/(A_0 kt + 1)$$

The last equation is a **hyperbolic** equation, and second-order, dimerization kinetics are often called hyperbolic kinetics.

The plots shown below are for a dimerization reaction with $[A_0] = 10^{-6}$ M and $k = 2 \times 10^5$ M^{-1} sec^{-1}. The decay in [A] with time (left panel) in this second-order reaction looks superficially similar to the first-order reaction described above but it is actually different as can be seen from the equations describing how [A] changes with time.
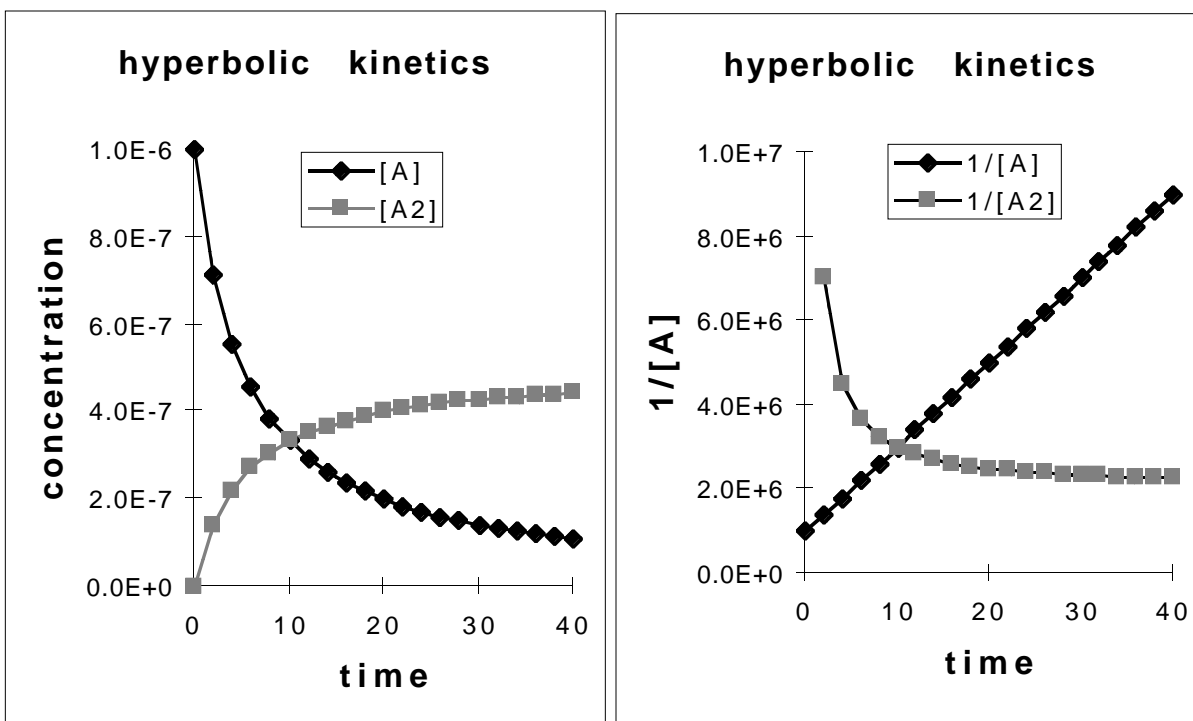
The simplest way to determine the rate constant in this case is to plot $1/[A]$ vs. time and determine the slope which is equal to k. Note, however, that plotting $1/[A_2]$ vs. time does not give a straight line. The expression for the half-life in this second-order case is:

$$t_{1/2} = 1/(k[A_0])$$

For the data shown, the half-life is 5 sec. It is important to note, however, that half-lifes for second-order reactions are fundamentally different than those for first-order reactions. In the first-order case, $t_{1/2}$ is independent of the starting concentration of the reaction. Here, the same reaction performed with different initial concentrations would have different half-lifes. Moreover, if it takes 5 sec for half of the molecules to react with a given initial concentration of A, then it will take 10 sec for half of the remaining molecules to react, etc. This makes intuitive sense because collisions between two A molecules will become less likely as the concentration of A decreases.

Bimolecular reactions with different reactants:

Reaction: $A+B \rightarrow AB$

Rate Law: $-d[A]/dt = -d[B]/dt = k[A][B]$

Integrated solution: COMPLICATED EXCEPT UNDER SPECIALED CONDITIONS

Because the general solution for this common class of bimolecular reactions is complicated, three specialized ways are generally used to determine the second-order rate constant.

(1) Mix A and B at time zero and estimate the initial rate of the reaction at different reactant concentrations. At t=0, $[A]=[A_0]$ and $[B]=[B_0]$, and the rate constant can therefore be calculated as:

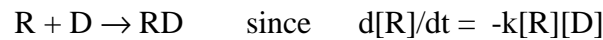$$k = (\text{initial rate})/([A_0][B_0])$$

(2) Arrange the experiment so that $[A_0] = [B_0]$. Now the integrated solution is identical to that for the dimerization reaction shown above and the rate constant can be obtained from a plot of $1/[A]$ or $1/[B]$ vs. time.

(3) Arrange the experiment so that $[A_0] \gg [B_0]$ or vice versa. If A is in large excess over B, then the concentration of $[A]$ will not change significantly during the reaction and the rate law can be written as:

$$-d[B]/dt = k[A_0][B] = k_{apparent}[B] \qquad \text{where } k_{apparent} = k[A_0]$$

Notice that this rate expression now has the same form and same integrated solution as a first-order reaction. We could now plot ln[B] vs. time and get a straight line with a slope equal to $-k[A_0]$. By manipulating the starting concentrations, we've made a bimolecular reaction into a **pseudo-first-order reaction**. Unlike a real first-order reaction, however, we would not get the same value of $k_{apparent}$ in experiments performed with different initial concentrations of $[A_0]$.

Testing Reaction Mechanisms: One of the uses of kinetics in biochemistry to establish which model best represents a reaction of interest. Say, for example, that we have a monomeric DNA-binding protein (R) that binds to a 20 bp fragment of DNA (D) but we don't know whether the protein binds as a monomer or dimer. If we measure the initial rate of the association reaction and find that it varies linearly with [R], then the preferred model would be:

$$R + D \rightarrow RD \qquad \text{since} \qquad d[R]/dt = -k[R][D]$$

Conversely, if we measure the initial rate of the association reaction and find that it varies in proportion to $[R]^2$ then this would support a model like:

$$2R + D \rightarrow R_2D \qquad \text{since} \qquad d[R]/dt = -k[R]^2[D]$$
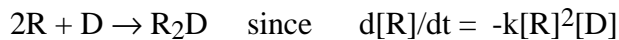
Kinetic experiments, by themselves, often can be used to rule out certain models but can't distinguish between other models that predict the same concentration dependence of the rates. In the example above, for instance, we specified that the free DNA binding protein was monomeric. What if we didn't know the oligomeric form of the protein in the free state? Now, a linear change in the initial association rate with increasing initial protein concentration could be consistent with reaction models such as:

$$R + D \rightarrow RD; \text{ or } R_2 + D \rightarrow R_2D; \text{ or } R_4 + D \rightarrow R_4D; \text{ etc.}$$
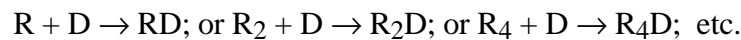
In this case, additional structural information about the oligomeric form of the free and/or bound proteins would be required to distinguish between the different reaction models.

POSSIBLE POINT OF CONFUSION: Reactions like $2R + D \rightarrow R_2D$; $2R_2 + D \rightarrow R_4D$; and $2R_4 + D \rightarrow R_8D$ all predict the same quadratic dependence of the association rate on protein concentration because, in each case, two free molecules of the protein combine in the complex. Similarly, $R + D \rightarrow RD$; $R_2 + D \rightarrow R_2D$; and $R_4 + D \rightarrow R_4D$ all predict the same linear dependence of the association rate on protein concentration because there is no change in the oligomeric form of the protein in going from the free to the bound species. ***So the key issue for kinetics is not whether an oligomer is bound but whether there is a change in oligomeric form during the binding reaction.***

Multi-step reactions: Reactions frequently do not occur in a single step but rather involve one or more intermediates. Consider the $2R + D \rightarrow R_2D$ reaction discussed above. It is unlikely that three molecules (2 proteins and 1 DNA site) will happen to collide at the same instant in solution to form the termolecular complex. There are two distinct ways in which assembly of the R_2D complex might occur by successive bimolecular reactions.

$$2R + D \rightarrow R_2 + D \rightarrow R_2D \quad \textbf{and/or} \quad 2R + D \rightarrow RD + R \rightarrow R_2D$$

which are shown schematically in the diagram below.
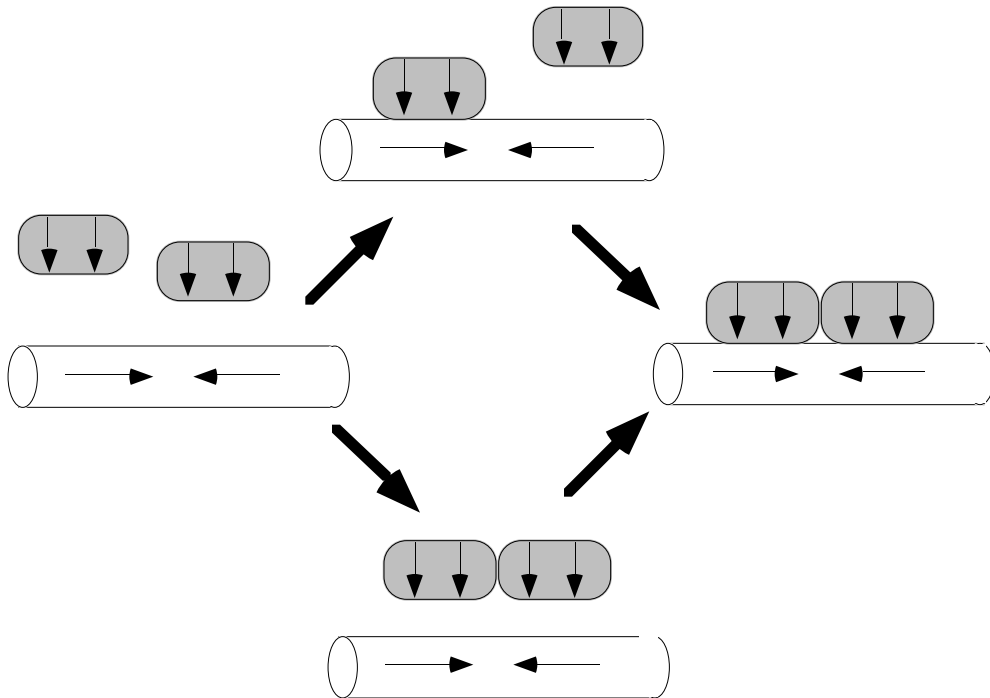
These mechanisms need not be mutually exclusive but if only one or the other represented the actual assembly pathway what kinds of experiments could help decide between these

models? One would be the identification of the predicted intermediates. Does the protein form a dimer in the absence of DNA at sufficiently high concentrations? Is a species with the electrophoretic behavior expected for RD seen in a gel-shift experiment? Detection of R_2 or RD species, by itself, wouldn't prove that these species participate as **on-pathway intermediates** in the reaction. For example, we've drawn the DNA-bound dimer as:
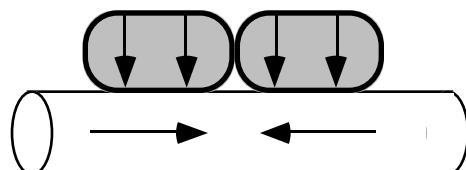
but maybe the R_2 and RD intermediates species have structures like:
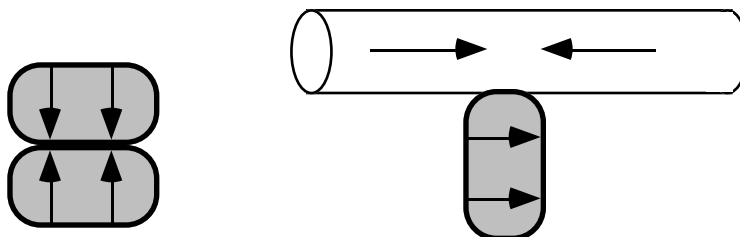
Such species might form but be **off-pathway** and have no relevance to the actual assembly pathway. If the R_2 and RD species were shown to have more reasonable structures, such as
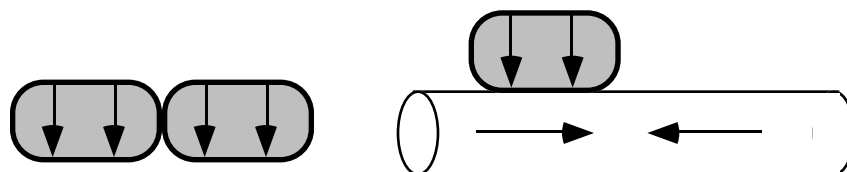
then this would make one feel better about the possibility that they were assembly intermediates but wouldn't necessarily be a guarantee.

The **rule of kinetic significance** states that for a species to be a bona fide intermediate in a reaction, it must form and disappear at a rate at least as fast as the final product. Thus, if either the R_2 or RD species were detected in the reaction but appeared at a rate slower than the rate of R_2D formation, then this would rule them out as significant intermediates in the major assembly pathway. Similarly, if R_2 were an authentic intermediate, then addition of D to purified R_2 should result in formation of R_2D at a rate at least as fast as its formation from 2R and D.

Rate-determining steps: In multistep reactions, one elementary step is often much slower than the other steps and becomes rate determining. That is, the rate of the overall

reaction is essentially determined by the rate of this slowest step. In such cases, speeding up the other elementary reactions would have a negligible effect on the overall rate of the reaction. In biological systems, rate-determining steps are usually the steps that are subject to regulation.

In the unimolecular example shown below, the rate constant for the B→C step is much smaller than the rate constant for the A→B step. Thus, B→C is the slow elementary step and is rate determining.

$$A \xrightarrow{20 \ s^{-1}} B \xrightarrow{1 \ s^{-1}} C$$

When we write reactions like this one, without the back reactions, we are assuming that the rate constants for these back reactions are small relative to the forward rate constants. In this model, if we start this reaction with pure A, what will happen? A will be rapidly converted to B but because B is converted to C more slowly, the concentration of B will build-up, as shown below, and C will appear with much slower kinetics.
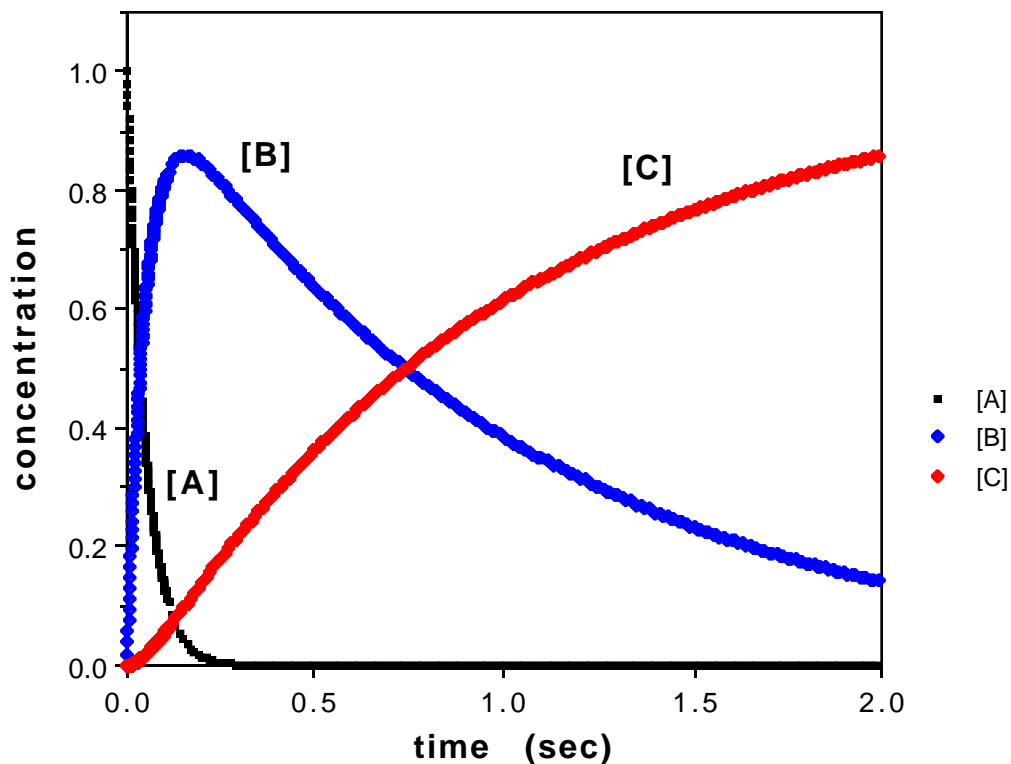
Here, B clearly satisfies the law of kinetic significance since it forms and disappears faster than the rate at which C appears. Notice also that formation of C occurs with a half life ≈ 0.7 sec and a rate of roughly 1 sec^{-1}, the magnitude of the rate constant for the slowest elementary step.

Now consider the same reaction but with the rate constants interchanged.

$$A \xrightarrow{1 \text{ s}^{-1}} B \xrightarrow{20 \text{ s}^{-1}} C$$

Now the A→B step is rate limiting. Once B is formed, it is rapidly converted to C and, as shown below, relatively little of the B intermediate ever accumulates. In general, intermediates only accumulate to significant extents when they precede a slow step or a rate determining step in the overall reaction.
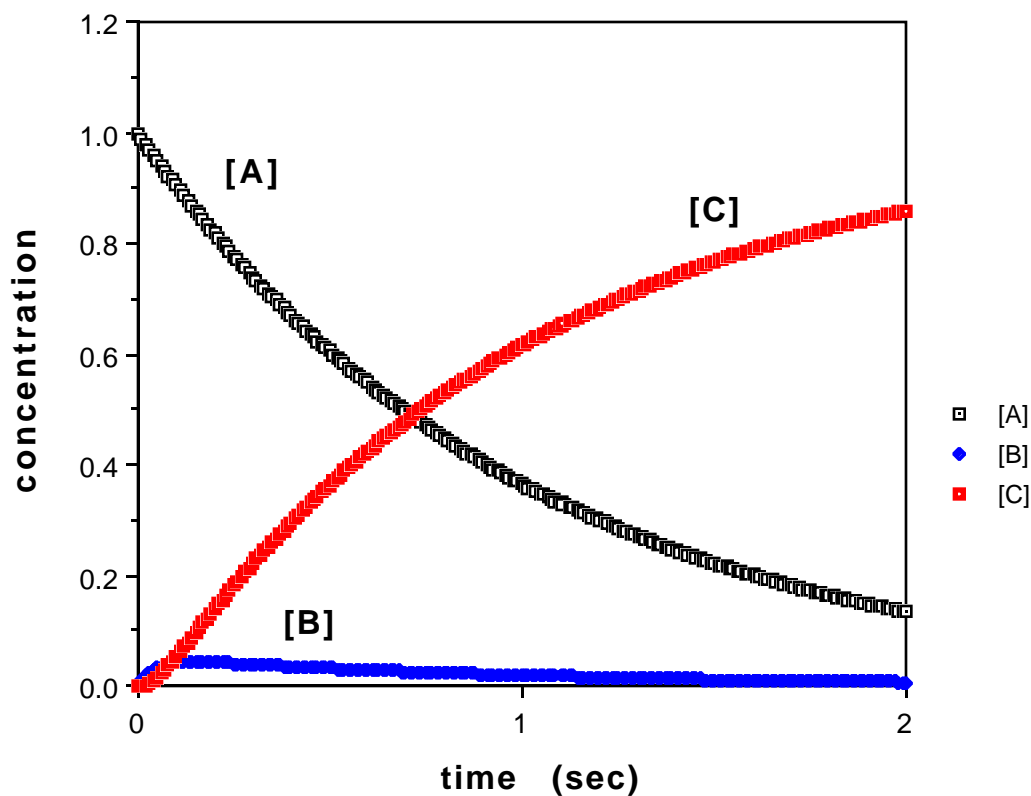
In this reaction, B also forms faster than C early in the reaction (satisfying the rule of kinetic significance) but it might be difficult to show this experimentally without very sensitive assays for low amounts of B and C. Again, note that formation of C occurs with a half life ≈ 0.7 sec and a rate of roughly 1 sec^{-1}, which is the rate constant for the slowest elementary step.

Rate-determining pre-equilibrium: So far, we've been largely dealing with reactions that only proceed in a single direction. A common class of kinetic reactions involve situations such as:

$$A \underset{200 \text{ s}^{-1}}{\overset{1 \text{ s}^{-1}}{\rightleftarrows}} B \xrightarrow{20 \text{ s}^{-1}} C$$
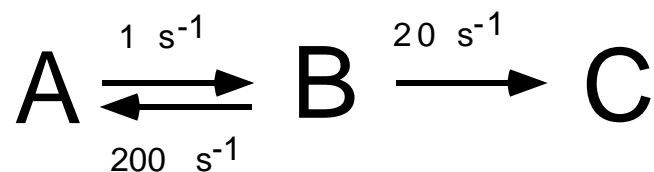
In this case, once a molecule of B is formed it can either convert back to A or go on to form C. With the rate constants shown, it should be clear that most B will revert to A because the rate constant for this step is 10-fold greater than the rate constant for conversion of B→C. Thus, it should take about 10 times longer in this model to form as much C as in the model where the B→A conversion was not allowed.

In such systems, A and B effectively equilibrate.

$$K_{ab} = [A]/[B] = (200 \text{ s}^{-1})/(1 \text{ s}^{-1}) = 200.$$

so there will never be much B relative to A. The rate at which C forms is just $20 \text{ s}^{-1}[B]$. Substituting for [B] gives

$$d[C]/dt = (20 \text{ s}^{-1})/(200) [A] = 0.1 \text{ s}^{-1} [A]$$

Thus, the half-life for formation of C is now about 7 sec, whereas it was 0.7 sec when the B→A conversion was not permitted. This is the same result as we arrived at above just by considering the rates at which B would partition between A and C. Notice that for this case, the overall reaction is slower than any of the elementary steps.

The case discussed above is a specific example of the general reaction:

$$A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} B \overset{k_2}{\longrightarrow} C$$
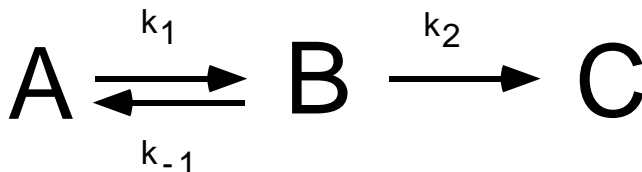
In such reactions, if $k_{-1} \gg k_2$ (B forms A much faster than it forms C), then there will be a rate-determining pre-equilibrium and the overall rate of formation of C will depend on all three rate constants.

$$d[C]/dt = (k_1 k_2)/(k_{-1}) [A] \qquad \text{equation 3.1}$$

if we assume, instead, that $k_2 \gg k_{-1}$ (B forms C much faster than it forms A), then we can effectively ignore the back reaction of B→A and the only question is whether k_1 or k_2 is rate-limiting.

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Steady-state approximation: *The kinetics of reactions of this type can also be also analyzed by writing the overall expression for the rate of change of B and setting this equal to 0. By doing this, we are making the assumption that the concentration of B rapidly reaches a constant, steady-state value that does not change appreciably during the reaction.*

$$d[B]/dt = k_1[A] - k_{-1}[B] - k_2[B] \; = \; k_1[A] - (k_{-1}+k_2)[B] = 0$$

$$(k_{-1}+k_2)[B] \; = \; k_1[A]$$

$$[B] = (k1)/(k_{-1} + k_2) \, [A]$$

substituting into $d[C]/dt = k_2[B]$ gives:

$$d[C]/dt = (k_1 k_2)/(k_{-1} + k_2) \, [A]$$

now if $k_{-1} >> k_2$

$$d[C]/dt \approx (k_1 \, k_2)/(k_{-1}) \, [A] \qquad \text{this is the same as eq. 3.1}$$

by contrast, if $k_2 >> k_{-1}$

$$d[C]/dt \approx (k_1 k_2)/(k_2) \, [A] \; = \; k_1[A] \qquad \text{equation 3.2}$$

Equation 3.2 is equivalent to saying that the k_1 step is rate determining for the overall reaction.

The steady-state approximation for an intermediate will not apply whenever the intermediate is formed at a rate much faster than its breakdown   For example, with the rate constants shown below
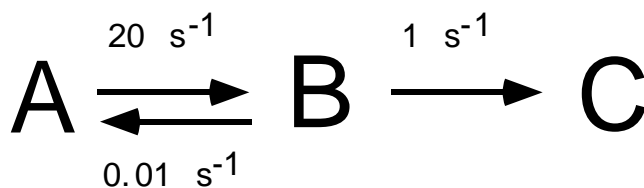
B forms much faster than it disappears and the concentration of B will build up rather than reaching a steady state. (Note that the kinetics here should be very similar to those graphed above on page 12). Most B will go on to form C rather than go back to A and thus the overall reaction should proceed with a rate constant of about 1 sec^{-1}. Even though $k_2 >> k_{-1}$ using equation 3.2 (which is only true if $d[B]/dt \approx 0$) in this instance would give the wrong answer because the steady-state assumption is invalid.

These general ideas about rapid pre-equilibrium and the steady-state approximation for intermediates are equally applicable to reactions in which the first step is bimolecular. For example,

$$A + B \underset{200 \ s^{-1}}{\overset{10^6 \ M^{-1} \ s^{-1}}{\rightleftharpoons}} AB \xrightarrow{20 \ s^{-1}} AC$$
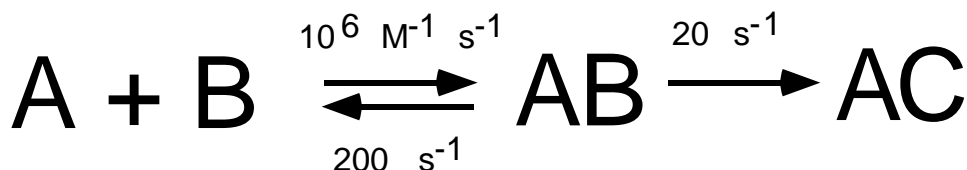
With the rate constants shown, AB will dissociate to A+B at a rate 10-times faster than it isomerizes to AC. Compared to a reaction in which the rate of AB dissociation was negligible, this will slow the formation of AC by a factor of approximately 10. We could again make the steady-state approximation for [AB] and derive the following expression.

$$d[AC]/dt \ = \ (k_1 k_2)/(k_{-1} + k_2) \ [A][B] \ \approx \ (k_1 k_2)/(k_{-1}) \ [A][B] \quad since \ k_2 \gg k_{-1}$$

We'll see expressions like this later when we deal with certain types of enzyme catalyzed reactions. Again, we would expect the steady state assumption to break down in the general case when the rate of formation of AB is much faster than its rate of breakdown. This will depend both on the rate constants and on the concentrations of A and B.

When intermediates can't be detected directly: Sometimes, intermediates in a reaction can't be isolated as distinct molecular species or characterized directly. In such cases, the existence of intermediates is often inferred from a lag phase in the kinetics, a burst phase in the kinetics, or from other types of multiphasic behavior (for example, two exponential phases are required to fit the observed data for a unimolecular reaction).

However, even when the kinetics of a unimolecular reaction are fit well by a single exponential phase, the absence of detectable intermediates can not be used as proof that intermediates don't exist. Rather, it means that if intermediates do exist, they must break down at rates significantly faster than the rates at which they form. This, in turn, generally means that they are unstable relative to both the reactants and products of the reaction. For example, during some protein folding reactions the only detectable populated species are the fully denatured protein or the fully native protein. In such a case, it seems unlikely that a disordered polypeptide chain could form the native protein directly in a single step since this would imply that several hundred native bond angles were correctly set simultaneously. This is probably a case in which whatever folding intermediates do exist are unstable and therefore don't accumulate during the course of the reaction.
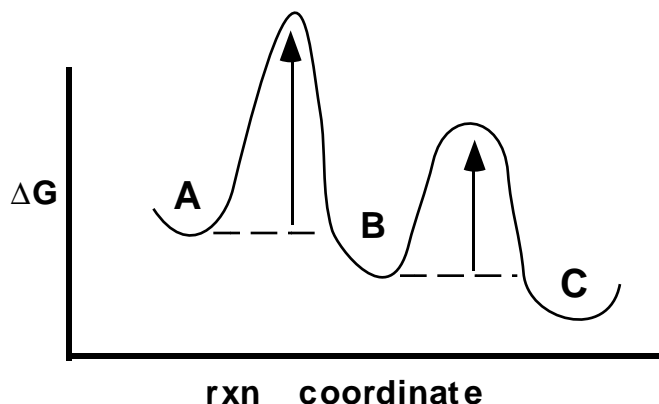
Reaction coordinate diagrams for reactions with intermediates: As noted above, when we write a multistep reaction that proceeds in a single direction, we're simply ignoring the back reactions which is justified if these reactions have much smaller rate

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constants than the forward reactions. The reaction coordinate diagram for such a situation might look like

$$A \xrightarrow{20 \ s^{-1}} B \xrightarrow{1 \ s^{-1}} C$$
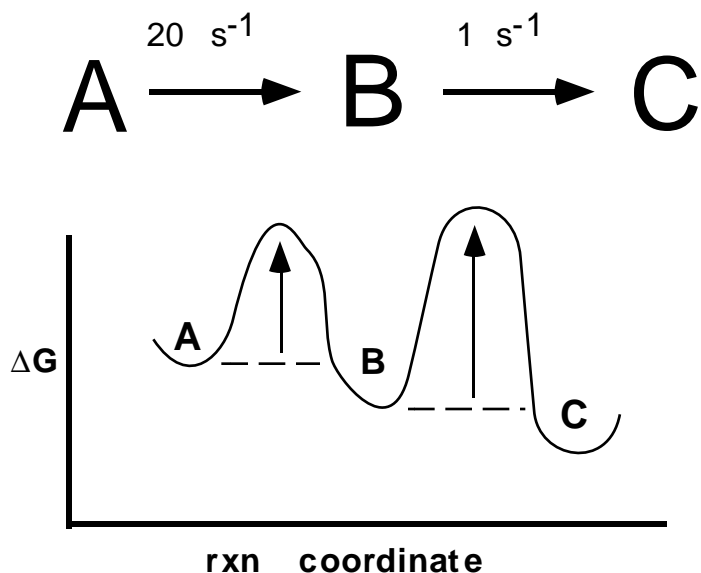
Notice that the free energy of A is higher than B which is higher than C. The reaction proceeds downhill. Also, the barrier between B and C is the highest, indicating that this is the slowest step. Finally, B is located at the bottom of a fairly deep valley, an indication that it should accumulate.

In the next reaction coordinate diagram, the first barrier has become the highest corresponding to the slowest step in the reaction. B is now in a shallower valley than before and so less will accumulate as an intermediate.

$$A \xrightarrow{1 \ s^{-1}} B \xrightarrow{20 \ s^{-1}} C$$

ΔG

A

B

C

rxn coordinate

If we now explicitly consider a rapid back reaction of B→A, we might get a reaction coordinate diagram that looks like:

$$A \underset{200 \ s^{-1}}{\overset{1 \ s^{-1}}{\rightleftharpoons}} B \xrightarrow{20 \ s^{-1}} C$$

ΔG

A

B

C

rxn coordinate
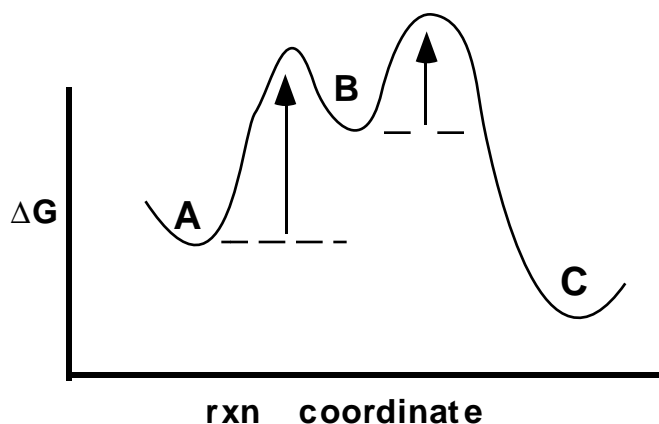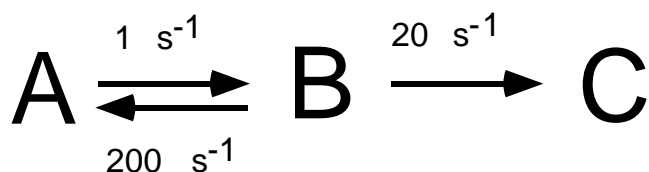
The first barrier still corresponds to the slowest elementary step because the distance from A to the first barrier peak represents the largest free energy change. B, however, is now less stable (has a higher free energy) than A. Once B is reached, the back reaction to A is kinetically preferred over the forward reaction to C because the barrier in the backward direction is lower than the one in the forward direction. In cases, like this one, where there is a rate determining pre-equilibrium, the highest point on the overall free energy landscape

(now the barrier peak between B and C) does not represent the slowest elementary step but does represent the rate-determining step of the overall reaction.

Consider, for example, what would now happen if we made the rate constant for the B→C conversion 100-fold faster.

$$A \underset{200 \ s^{-1}}{\overset{1 \ s^{-1}}{\rightleftharpoons}} B \xrightarrow{2\,000 \ s^{-1}} C$$

rxn   coordinate
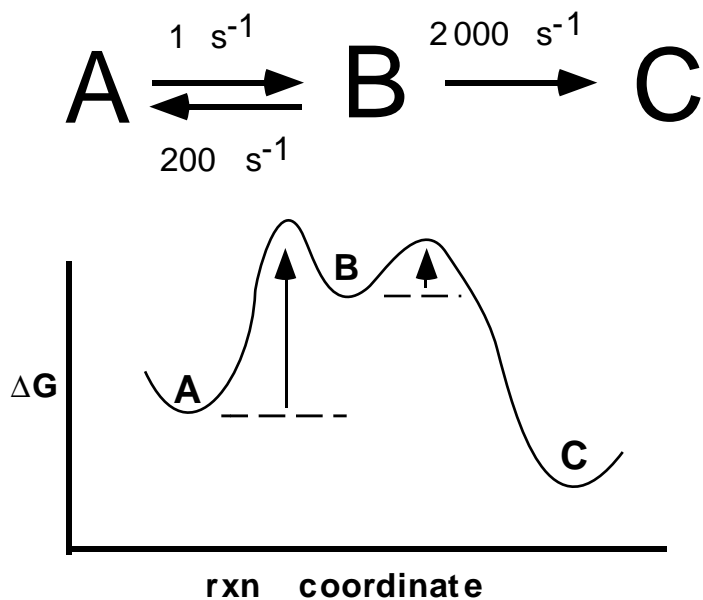
Now, once a molecule reaches B, it will go on to form C most of the time. The barrier between A and B has again become rate determining. By speeding up the B→C reaction, the entire reaction now goes faster because there is no longer a rate-determining pre-equilibrium. This is a case where regulation of a kinetic step that is not the slowest elementary step could have significant kinetic consequences on the rate of the overall A→C reaction.