

THE KINETICS OF CHEMICAL REACTIONS: SINGLE-MOLECULE VERSUS “BULK” VIEW

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The single experience of one coin being spun once has been repeated a hundred and fifty six times. On the whole, doubtful. Or, a spectacular vindication of the principle that each individual coin spun individually is as likely to come down heads as tails and therefore should cause no surprise each individual time it does

Tom Stoppard, “Rosencrantz and Guildenstern are Dead”

Chemistry is a discipline that studies how molecules convert into one another. A chemical reaction can involve several different molecules. For example, a molecule of sodium chloride can fall apart (i.e., dissociate) into the sodium and chlorine atoms. The simplest possible chemical reaction involves conversion of some molecule A into a different form B. To describe this reaction, we write the following equation:



Generally, whatever is on the left hand side of the equation is called the “reactant(s)” and whatever is on the right is the “product(s)”. So, in our example, A is the reactant and B is the product. Since no other molecules are involved in this process, A and B must consist of the same atoms. That is, they must be described by the same chemical formula. The only difference between A and B can then be in the manner in which those atoms are arranged in the molecule. Figure 1 shows two examples: In the first one, we have the molecule of 1,2-dichloroethylene, which is described by the chemical formula $C_2H_2Cl_2$. This molecule can exist in two possible conformations (called isomers), which are our molecules A and B. The conversion between A and B in this case is what chemists refer to as isomerization. The second example involves a much more complicated process, in which a disordered molecular chain A undergoes

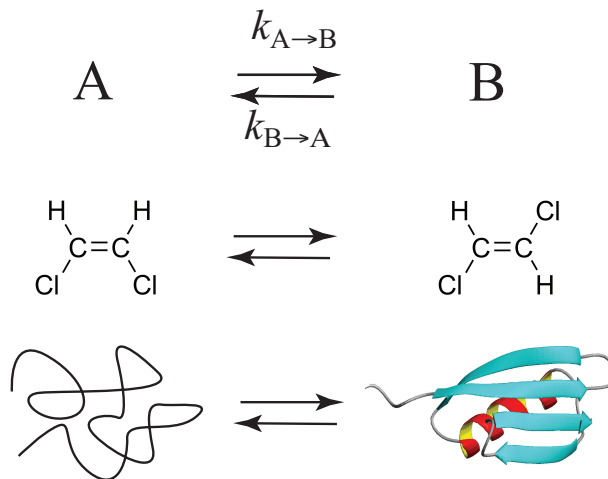


FIGURE 1. Examples of a reversible reaction $A=B$: isomerization of a small molecule and protein folding/unfolding. The second process involves self-structuring of a long molecular chain, typically consisting of hundreds or thousands of atoms, which ends with a folded protein molecule. The atomistic details are not shown in the second case.

a rearrangement where it forms a well defined structure, the protein molecule B. This process is commonly referred to as protein folding, where A represents the unfolded protein state and B is the folded one. Biological molecules, such as proteins and RNA, have to fold in order to function, and so the processes of this type are of great interest to biophysicists and biochemists.

At this point you may ask: Why only two states, A and B, are used to describe this process? Clearly, there is a continuum of different shapes that a molecule can assume. For example, it seems likely that to go from one planar configuration in Fig. 1 to the other, the molecule $C_2H_2Cl_2$ must be twisted 180 degrees around the $C = C$ bond, thus requiring it to assume a series of non-planar shapes along the way. Perhaps then a reaction of the form $A = I_1 = I_2 = \dots = B$, where I_n represents all those non-planar intermediates (the number of which is, strictly speaking, infinite), would make more sense. Likewise, in our second example the unfolded protein state A does not even represent any unique molecular arrangement. Rather, A describes a collection of random-looking molecular shapes each resembling a long piece of spaghetti. Why do we lump all those into a single state? These are very interesting questions. The fundamental reasons why a collection of states can, in certain cases, be represented as a single state will be explained later on. For now let us adopt a more pragmatic view that based on how different molecular configurations are observed in practice. A chemist tells the difference between A and B by observing some physical property

of the molecule. For example, A and B may absorb light at different wavelengths, i.e., they have different colors. If only two colors can be experimentally observed, we call the chemical containing the molecules of one color A and the other color B. If more colors are seen, then we need more states, or molecular species, to describe the process. It turns out that in many cases only two colors, rather than a continuum of colors, are observed. Whenever this is the case we use our reaction scheme (1).

There is another empirical observation that will have to be adopted here (and will be justified later). It is quite common for the reactions described by Eq.(1) that if one mixes N_A molecules of A and N_B molecules of B, then these numbers will evolve in time according to the following differential equations

$$\begin{aligned}\frac{dN_A}{dt} &= -k_{A\rightarrow B}N_A + k_{B\rightarrow A}N_B \\ \frac{dN_B}{dt} &= -k_{B\rightarrow A}N_B + k_{A\rightarrow B}N_A\end{aligned}\tag{2}$$

Chemists call the time evolution described by these equations “first order kinetics”. These equations are not exact. Indeed, they cannot be exact since they describe continuously changing functions while the number of molecules is an integer number. Nevertheless, as long as the total number of molecules in the system is very large, they are a reasonable approximation. The quantities $k_{A\rightarrow B}$ and $k_{B\rightarrow A}$ are called the *rate constants* or *rate coefficients* for going from A to B or B to A. They are not really constants in that they typically depend on the temperature and, possibly, on other properties of the system. Nevertheless, as silly as the phrase “temperature dependence of the rate constant” sounds, this is something you will have to get used to if you hang around chemists long enough.

Let us now examine what the solution of Eq.(2) looks like. Suppose that we start with molecules of A only and no molecules of B. For example, we could heat up our solution of proteins, which typically results in all of them being unfolded; then we could quickly reduce the temperature of the solution and watch some of them fold. That is, our initial condition is $N_A(0) = N$, $N_B(0) = 0$. The solution is then given by:

$$\begin{aligned}N_B(t) &= N \frac{k_{A\rightarrow B}}{k_{A\rightarrow B} + k_{B\rightarrow A}} (1 - e^{-(k_{A\rightarrow B} + k_{B\rightarrow A})t}) \\ N_A(t) &= N - N_B(t)\end{aligned}\tag{3}$$

and is schematically shown in Figure 2.

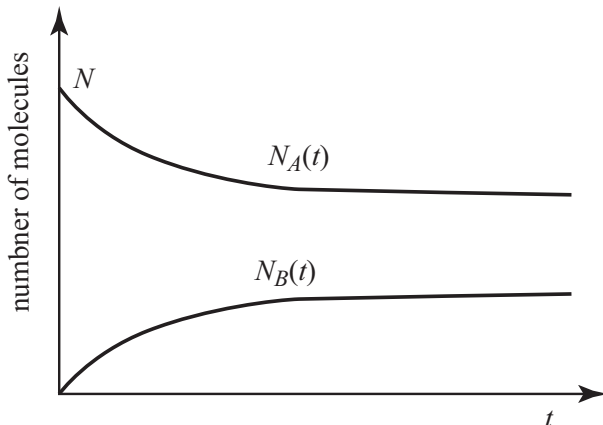


FIGURE 2. Starting with the pure reactant A, the amount of the reactant decreases and the amount of the product B increases until they reach their equilibrium values.

We see that, at first, the number of molecules of A decreases and the number of molecules B increases. Eventually, as $t \rightarrow \infty$, these amounts attain constant values, $N_A(\infty)$ and $N_B(\infty)$. This situation is referred to as *chemical equilibrium*. The equilibrium amounts of A and B satisfy the relationship

$$k_{A \rightarrow B} N_A(\infty) = k_{B \rightarrow A} N_B(\infty), \quad (4)$$

which, according to Eq. 2, ensures that the equilibrium amounts stay constant, $dN_A/dt = dN_B/dt = 0$.

Imagine now that we can see what individual molecules are doing. According to our initial condition, at $t = 0$ they are all in state A. Eq. 3 then predicts that the population of molecules in A will start decreasing as a function of time, following a nice smooth curve such as the one shown in Fig. 2. How can we interpret this behavior in terms of the underlying behavior of each individual molecule? Perhaps the molecules, initially all in state A, are “unhappy” to be so far away from the equilibrium situation and so they are all inclined to jump into B, in an organized fashion, until sufficient number of molecules B are created. This, however, seems highly unlikely. Indeed, for this to happen the behavior of a molecule should be somehow influenced by the number of other molecules in the states A and B. Molecules interact via distant-dependent forces. It is, of course, not inconceivable that interaction between two neighboring molecules could make them undergo a transition to another state in a concerted fashion. However this can only happen when the molecules are close enough. If the experiment is performed in a large beaker, the behavior of two sufficiently distant molecules should be uncorrelated. Moreover,

any cooperativity among molecules would disappear as the number of molecules per unit volume becomes smaller and smaller and, consequently, the average distance between the molecules increases. Since Eq. 2 is known to be satisfied even for very dilute solutions of molecules, the hypothesis that the molecules somehow conspire to follow these equations must be wrong.

On the contrary, Eq. 2 is consistent with the view that the molecules are entirely independent of one another and each is behaving in a random fashion! Indeed, consider Figure 3, where we have $N = 6$ molecules (the trajectories of only 3 of them are shown). Each molecule jumps randomly between the states A and B. I have generated a trajectory for each on the computer using a simple random number generator (a computer program that spits out random numbers). Of course I had to know some additional information: For example, it is necessary to know something about the average frequency of the jumps. The precise mathematical description of the random jump process will be given below.

The trajectory of each molecule was generated independently. The molecules, however, were "synchronized" at the beginning of the calculation. That is, they are all in state A at $t = 0$. If we wait for some time, some of them will make a transition to B so that N_A will decrease (and N_B will increase). Indeed, such a decrease is observed in Fig. 3. Although the shape of the curve $N_A(t)$ is somewhat noisy, it is close to the curve obtained from Eq.3. If I repeat this calculation (i.e., generate new random-jump trajectories for each molecule) I will get another $N_A(t)$ curve. Most of the time, it will be close to Eq.3 although, occasionally, significant deviations may be observed. The noise will however go away if we repeat the same experiment with a much larger number of molecules. Even though each individual molecule is random, a large collection (or, as physicists call it, an *ensemble*) of such molecules behave in a predictable way. Such a predictable behavior is not unusual when large numbers of randomly behaving objects are involved. Your insurance company, for example, cannot predict each individual car accident occurring to their clients yet they can estimate the total number of accidents. They would not know how much to charge you for your policy otherwise. Likewise, although each molecule of air is equally likely to be anywhere in your house, you probably do not need to worry about suffocating in your sleep as a result of all the molecules gathering in the kitchen.

Our picture of randomly behaving, independent molecules also explains the origin of chemical equilibrium in the system. At $t = 0$ all the molecules are in state A. Since there are no molecules in state B, the only transitions we will see early on are those from A to B. As the time goes by, there will be more molecules in B and, consequently, transitions from B to A will begin to occur. Once the number of A \rightarrow B transitions is, on the average, balanced by that of B \rightarrow A transitions, the total

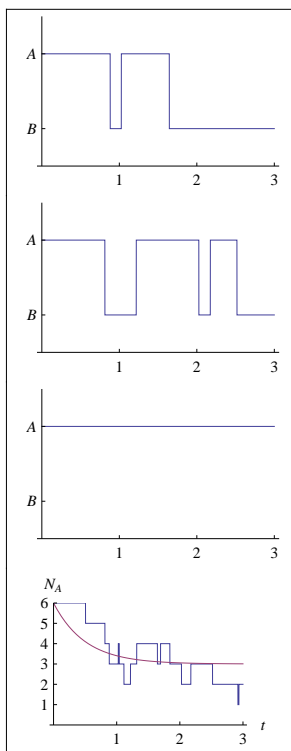


FIGURE 3. Six molecules were initially prepared in the reactant state (A). Each molecule proceeds to jump, in a random fashion, between A and B. Only the trajectories of three of them are shown. This results in a decaying population of state A, N_A , which agrees with the prediction of Eq.3 shown as a smooth line

number of molecules in A or B will stay nearly constant. Equilibrium, therefore, results from a dynamic balance between molecules undergoing transitions in both directions.

Let us emphasize that equilibrium here is a property of the *ensemble* of the molecules, not of each individual molecule. That is, each molecule does not know whether or not it is in equilibrium. In other words, if we were to examine the sequence of random jumps exhibited by any specific member of the ensemble, it would not appear in any way special at $t = 0$ or at any value of time t . The nonequilibrium situation at $t = 0$ results from synchronization of all the molecules, i.e., forcing them all to be in state A. This creates a highly atypical (i.e., nonequilibrium) state of the entire ensemble for, while there is nothing atypical about one particular molecule being in state A, it is highly unlikely that they all are in A, unless forced to by the

experimentalist. Starting from this initial state, each molecule proceeds to evolve randomly and independently of other molecules. At sufficiently short times t , there still a good chance that many molecules have remained in the initial state A so they are correlated, not because they interact with one another but because they have not all forgotten their initial state. Once all of them have had enough time to make a few jumps, they are all out of sync (see Fig. 3), any memory of the initial state is lost, and the ensemble is in equilibrium.

So far I have been vague about the specifics of the random-jump process exhibited by each molecule. To develop a mathematical description of this process, divide Eq.2 by the total number of molecules $N = N_A + N_B$. As our molecules are independent, it then makes sense to interpret $p_{A,B} = N_{A,B}/N$ as the probabilities to be in states A or B. This gives

$$\begin{aligned}\frac{dp_A}{dt} &= -k_{A \rightarrow B}p_A + k_{B \rightarrow A}p_B \\ \frac{dp_B}{dt} &= -k_{B \rightarrow A}p_B + k_{A \rightarrow B}p_A\end{aligned}\tag{5}$$

According to these equations, if the time is advanced by a small amount δt then the probability of being in A will become:

$$p_A(t + \delta t) = (1 - k_{A \rightarrow B}\delta t)p_A(t) + (k_{B \rightarrow A}\delta t)p_B(t)\tag{6}$$

We can interpret this as follows:

$$p_A(t + \delta t) = (\text{conditional probability to stay in A having started from A}) \times (\text{probability to start in A}) + \\ + (\text{conditional probability to make transition from B to A}) \times (\text{probability to start in B})$$

Therefore if the molecule is found in state A, the probability for it to make a jump to B during a short time interval δt is equal to $k_{A \rightarrow B}\delta t$. The probability that it will stay in A is δt is $1 - k_{A \rightarrow B}\delta t$. The rate constant $k_{A \rightarrow B}$ is therefore the probability of the reaction $A \rightarrow B$ happening per unit time.

By advancing the time in small steps δt and deciding whether or not to jump to the other state as described above, a single-molecule trajectory can be created, which corresponds to specified values of the rate constants $k_{A \rightarrow B}$ and $k_{B \rightarrow A}$. This way, you can generate your own version of Figure 3. Suppose, for example, that at $t = 0$ the molecule is in A. Advance time by δt . Now chose the new state to be B with the probability $k_{A \rightarrow B}\delta t$ and A with the probability $1 - k_{A \rightarrow B}\delta t$. And so on. Whenever the molecule is in state B, upon advancement of time by δt the new state becomes A with the probability $k_{B \rightarrow A}\delta t$ and remains B with the probability $1 - k_{B \rightarrow A}\delta t$. This procedure creates a discrete version of the trajectory shown in Fig.4, with the state

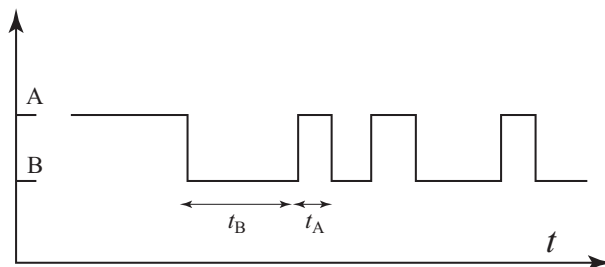


FIGURE 4

of a molecule specified at $t = 0, \delta t, 2\delta t, \dots$. It will become continuous in the limit $\delta t \rightarrow 0$.

A traditional chemical kineticist deals with curves describing the amounts of various molecules such as the ones shown in Fig. 2. Given the experimentally measured time evolution of $N_A(t)$ and $N_B(t)$, he or she can attempt to fit these dependences with Equations 2 or 3. If such a fit is successful, it will provide an estimate for the rate constants $k_{A \rightarrow B}$ and $k_{B \rightarrow A}$. In contrast, a single-molecule chemist is faced with a dependence like the one shown in Fig. 4. Because this dependence is a random process, it is not immediately obvious how our single-molecule kineticist could extract the rate constants from the experimental data. It turns out that Fig. 4 contains the same information as Fig. 2. To see this, consider the time t_A (or t_B) the molecule dwells in A (or B) before it jumps to the other state (Fig. 4). Let

$$p_{A \rightarrow B}(t_A) dt_A$$

be the probability that the molecule will make a transition from A to B between t_A and $t_A + dt_A$, where the clock measuring t_A was started the moment the molecule has made a transition from B to A. Recall that the rate constant $k_{A \rightarrow B}$ has been interpreted as the probability of making the transition to B per unit time. It is then tempting to write

$$p_{A \rightarrow B}(t_A) dt_A = k_{A \rightarrow B} dt_A$$

Unfortunately this is wrong. Indeed, integrating the above equation would lead to a cumulative transition probability to make the transition (at any time)

$$\int_0^{\infty} p_{A \rightarrow B}(t_A) dt_A = \infty$$

that is infinite. This is nonsense since probabilities cannot be infinite. Instead, a more reasonable expectation would be for the above integral to be equal to 1, which means that the transition happens with certainty provided one waits long enough.

What is wrong with our logic? Recall that $k_{A \rightarrow B} dt_A$ is a *conditional probability* of making a transition from A to B *provided that the molecule is found in state A at t_A* . But it is possible that the molecule has already made the transition before t_A . The correct equation to write is then:

$$p_{A \rightarrow B}(t_A) dt_A = k_{A \rightarrow B} dt_A S_A(t_A), \quad (7)$$

where $S_A(t_A)$ is the survival probability, i.e., the probability that the molecule has not escaped from A during the time interval between 0 and t_A . Because the only way to escape from A is to make a transition to B, it is clear that the transition probability $p_{A \rightarrow B}(t_A) dt_A$ is equal to the amount by which the survival probability decreases during dt_A , $-[S_A(t_A + dt_A) - S_A(t_A)] = -dS_A$. This leads to a differential equation for the survival probability,

$$-dS_A = k_{A \rightarrow B} S_A dt_A. \quad (8)$$

Solving this with the initial condition $S(0) = 1$ gives the survival probability

$$S_A(t_A) = \exp(-k_{A \rightarrow B} t_A) \quad (9)$$

and the transition probability

$$p_A(t_A) = k_{A \rightarrow B} \exp(-k_{A \rightarrow B} t_A) \quad (10)$$

As expected, this transition probability satisfies the condition $\int_0^\infty p_A(t_A) dt_A = 1$, which implies that, if one waits long enough, the molecule will undergo a transition to B, with certainty. “Long enough” means much longer than the average dwell time in A, which is given by

$$\langle t_A \rangle = \int t_A p_A(t_A) dt_A = 1/k_{A \rightarrow B} \quad (11)$$

The latter relationship shows that the rate constants are directly related to the mean dwell times spent to each state and so they can be inferred from a long trajectory such as the one shown in Figure 4. These dwell times further satisfy the relationship

$$\langle t_B \rangle / \langle t_A \rangle = k_{A \rightarrow B} / k_{B \rightarrow A} \quad (12)$$

Compare this with Eq. 4, which predicts:

$$\langle N_B(\infty) \rangle / \langle N_A(\infty) \rangle = k_{A \rightarrow B} / k_{B \rightarrow A} \quad (13)$$

The similarity between the two ratios is not surprising. Indeed, it sounds intuitively appealing that the equilibrium number of molecules found in state A should be proportional to the time each given molecule spends in this state. More generally, this is the *ergodicity principle* at work. This principle declares any time average performed over a long trajectory of a molecule should be identical to an average over an equilibrium ensemble of such molecules. Consider the equilibrium probability of being in state A, $p_A(\infty)$. It can be calculated as the ensemble average, i.e., the fraction of molecules found in A:

$$p_A(\infty) = \frac{N_A(\infty)}{N_A(\infty) + N_B(\infty)}$$

It can also be calculated as the time average, i.e., the fraction of the time the molecule spends in A:

$$p_A(\infty) = \frac{\langle t_A \rangle}{\langle t_B \rangle + \langle t_A \rangle}$$

In view of Eqs. 12 and 13, the two methods give the same result.

$$p_A(\infty) = \frac{k_{B \rightarrow A}}{k_{A \rightarrow B} + k_{B \rightarrow A}}$$

Although these findings are plausible, we note that there are many systems that do not behave in an ergodic way. We will see a few examples later.

Our example shows that a single-molecule measurement can provide the same information (i.e., the rate constants) as the traditional, bulk chemical kinetics. But if both provide the same information, why do single-molecule measurements? As discussed in the Introduction, single molecule experiments require rather involved microscopy and single-photon detection and so they can be difficult and expensive. Moreover, real single-molecule data rarely look like our idealized Figure 4. Rather, real single molecule trajectories are often plagued by noise and are also too short

to allow reliable statistical analysis required to accurately estimate the underlying kinetic parameters such as the rate coefficients.

The above are just a few reasons for the skepticism encountered by the single-molecule experimenters in the early stages of the field. There are however many advantages to measuring chemical kinetics the single-molecule way. Those advantages will become more clear later on in this book. The simple two-state example considered here, however, already reveals one important advantage. Suppose A converts to be much faster than B to A, i.e., $k_{A \rightarrow B} \gg k_{B \rightarrow A}$. Then, according to Eq.4, we have $N_A \ll N_B$ in equilibrium. What is we are specifically interested in the properties of the molecule A? As the equilibrium mixture of A and B mostly consists of B, whatever experimental measurement we choose to make will report mostly on the properties of B rather than A. If we want to specifically study A, first of all we will need to find a way to drive all of our molecules to the state A. Even if we have succeeded in doing so, we only have a very short time interval, $\Delta t \ll 1/k_{A \rightarrow B}$, before most of the molecules will jump back to B. A single-molecule approach resolves this problem: As we observe a molecule's trajectory, we know what state it is in. If we are specifically interested in A, then we simply interrogate the molecule while it is in A.

A related advantage of single-molecule experiments is that they do not require synchronization of a large number of molecules to study their time dependent properties. Indeed, in order to measure $k_{A \rightarrow B}$ and $k_{B \rightarrow A}$ using a bulk technique, it is necessary that one starts away from equilibrium. For example, to observe the curve of Eq.3 it is necessary to synchronize all the molecules by drive all of them to state A at $t = 0$. As mentioned earlier, this sometimes can be achieved by quickly changing the conditions of the experiment. For example, one can quickly change the temperature of the system. In doing so, it is necessary to ensure that the preparation process itself is much faster than the process one is trying to observe (e.g., the conversion of A to B and back to A). If, for instance, the rates $k_{A \rightarrow B}$ and $k_{B \rightarrow A}$ are of order of $10^3 s^{-1}$, then one will have to devise a way of heating the system up or cooling it down in less than a millisecond. This is not easy, especially if A and B are mixed in a beaker of a macroscopic size! Single-molecule experiments sidestep this difficulty altogether by observing the trajectories of molecules under equilibrium conditions, one molecule at a time.