

Simple Statistical Thermodynamics for the Biochemist

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The time-average structure [of a protein] observed by X-ray diffraction is something of an abstraction, since it is not itself widely—or even sparsely—represented at any given time in the population of molecules. I mean by this that if we were to take an instantaneous picture of the molecular population showing us all the coordinates of the atoms for each individual molecule we would have difficulty in finding one that will match the average in *all* respects, although *most* of the molecules will have *most* of the features in common with it. Indeed, the protein molecule model resulting from the X-ray crystallographic observations is a “platonic” protein, well removed in its perfection from the kicking and screaming “stochastic” molecule that we infer must exist in solution. The great importance of the former lies in that it has permitted us to see the origin of the “bulk properties” of the protein, which result from averaging over the whole population. However, when it comes to the conversion of one structure into another, and to render account of any dynamic function of the protein, consideration of the “stochastic” species is indispensable.

Gregorio Weber. (1975). “Energetics of ligand binding to proteins.” *Advances in Protein Chemistry* 29:64-65

1 Introduction

Biomolecules are tiny. This has two important implications for trying to understand how they work:

1. **The dominant force they experience is buffeting by water molecules.** When thinking about molecular motion, we should picture a mosh pit rather than a ballet. A molecule moves first this way, then that, then the other way in random fashion. Whatever amazing function they accomplish has to overcome (or utilize!) this inescapable randomness.
2. **We never study individual molecules, only statistical distributions of many molecules.** If we do an experiment using a $1\ \mu\text{L}$ drop of $10\ \mu\text{M}$ protein, we average over a million million protein molecules. Even single molecule techniques like cryo-EM microscopy or single-molecule FRET only become meaningful after thousands upon thousands of observations.

This leads to a fundamental fact: **Biochemistry is *stochastic*. It deals in probabilities, not individual molecules.** Whatever biochemical mechanisms we come up with must take this into account. A mechanism is a non-starter if it requires molecules moving smoothly through the cell, or ignores the incessant and overwhelming influence of water, or assumes that two molecules “know” that they need to “find” one another.

Fortunately, we have powerful theoretical, computational, and experimental tools for understanding biomolecules from a statistical perspective. Unfortunately, even foundational concepts in this discipline can be difficult to grasp. Although students encounter words like *entropy* and *enthalpy* and *heat* in first-year general chemistry, the deep meaning of these words is often elusive. This is, in part, because understanding these terms requires mastering ideas from statistical physics—a nontrivial task for a physics student, much less a molecular biologist.¹

But physicists aren’t the only scientists who deal in the mechanisms of biomolecules. Biochemists, cell biologists, molecular biologists, and geneticists must often evoke a molecular explanation to answer questions in their field. How can such scientists develop statistical and molecular intuition for these problems without being overwhelmed by statistical physics?

This document is one attempt to answer this question. We will attempt to develop a framework for non-physicists to think about biomolecules in statistical thermodynamic terms. We will use very simple models to explore key ideas in statistical thermodynamics. The framework we develop will remain imprecise. We will develop math to describe something specific—like dice, or toy protein models—and then baldly assert the result is general. The goal is to provide

¹In his excellent statistical thermodynamics book, Terrell Hill begins: “We introduce the principles of statistical mechanics...in quantum-mechanical language because the argument is not only more general but is actually much simpler this way.” Powerful? Yes. Generally accessible? No.

a flavor for such approaches, without getting bogged down in details. While rigor is important, it can also interfere with intelligibility, particularly for the non-specialist.

This document is no replacement of one of the excellent textbooks that delve into physical biochemistry. Hopefully the simple examples we discuss can whet your appetite to dive deeper into the subject. A few resources are “Biological Physics” by Phillip Nelson; “The Molecules of Life” by John Kuriyan, Boyana Konforti, and David Wemmer; “Elements of Statistical Thermodynamics” by John Nash; “Statistical Mechanics: A Concise Introduction for Chemists” by Benjamin Widom; “An Introduction to Statistical Thermodynamics” by Terrell Hill; and “Molecular Driving Forces: Statistical Thermodynamics in Biology” by Ken Dill and Sarina Bromberg.

With these preliminaries out of the way, let’s begin.

2 The Dice Folding Reaction:

For chemical reactions, we’re often interested in questions like: *What direction is favored?* and *How fast will the reaction occur?* To explore these questions, we will start with something we’ll call the Dice Folding Reaction (DFR). Imagine a large number of six-sided dice. These are all “molecules” that can each be in one of six different “conformations” (\square , \square , \square , \square , \square , or \square). We will consider the “reaction”:

$$\{\square, \square, \square, \square, \square\} \rightleftharpoons \square. \quad (1)$$

This is analagous to a protein folding reaction in which there is an unfolded state made up of many conformations and a folded state made up of one conformation. The “unfolded” dice are those showing \square , \square , \square , \square , or \square . The “folded” dice are those showing \square (Fig 1).

2.1 Definitions and assumptions:

1. We have N dice, with N_{\square} of them showing \square .
2. The probability of rolling \square is p_{\square} .
3. Time advances in discrete, uniform steps Δt .
4. For each Δt , randomly select a fraction of the dice (α) and then roll them. We will always select $\alpha \ll 1.0$.²
5. The dice are far enough apart from one another that they do not interact. (In chemical terms, we have “dilute” dice.)

²In case the notation is unfamiliar, \ll means “much less than.” We use $\alpha \ll 1$ to avoid the complexity of rolling the same dice twice in one turn. Imagine for a second that we selected $\alpha > 1$. This would mean that at least one dice gets rolled more than once in a turn. This can be dealt with, but makes the dynamics more complicated. If α is small, the odds of hitting the same dice over Δt are small and can be neglected. In terms of chemistry, a small α means we are taking very small time steps, so not much can happen per step.

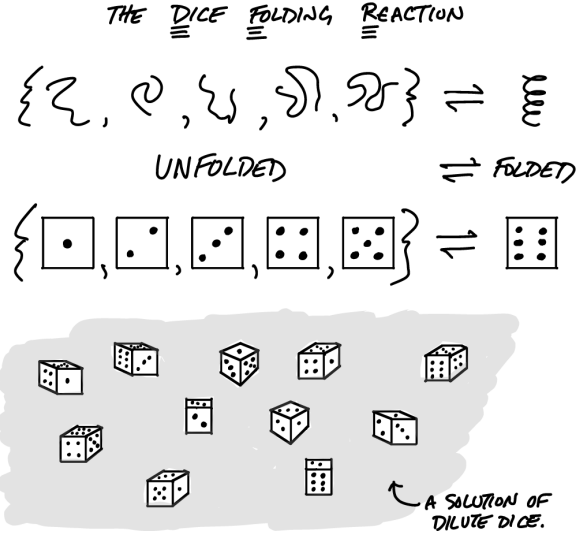


Figure 1: Elements of the Dice Folding Reaction

2.2 Dice dynamics

Remember, our goal is to use the DFR to ask questions about which direction in a reaction is favored (and why), as well as how fast a reaction runs. We'll start by thinking about the process by which dice showing M are gained and lost. We can model how many M are gained by:

$$\frac{N_{\text{other} \rightarrow \text{M}}}{\Delta t} = \alpha \cdot (N - N_{\text{M}}) \cdot p_{\text{M}}. \quad (2)$$

Before you read further, pause for a moment. Can you figure out what this equation does given the definitions of the terms above?

Don't worry, we'll wait.

Hopefully you came to something like this: The average number of new M after a step is determined by 1) how many dice we rolled that could become M and 2) the probability each becomes M . The number of dice that can become M is determined by what fraction of all dice we rolled (α) and how many dice were \square , \square , \square , \square , or \square ($N - N_{\text{M}}$). The probability a each yields M is simply p_{M} .

What about the number of dice that start as M but are lost after a step? We can model this by:

$$\frac{N_{\text{M} \rightarrow \text{other}}}{\Delta t} = \alpha \cdot N_{\text{M}} \cdot (1 - p_{\text{M}}) \quad (3)$$

This is directly analogous to the M gain case. The number of M lost per step is determined by the fraction of the dice we rolled (α), how many dice were M

($N_{\text{Ⓢ}}$), and the probability that each roll ends up as different than Ⓢ ($1 - p_{\text{Ⓢ}}$). An example of the model in action is shown in Fig 2.

2.3 A quick aside on how to cheat at dice

Before moving on, let's pause to make sure we understand what $p_{\text{Ⓢ}}$ means. (This will come in handy later). When we roll a dice, it can come up in one of six ways. With this in mind, a very general way to write $p_{\text{Ⓢ}}$ is:

$$p_{\text{Ⓢ}} = \frac{w_6}{\sum_{i=1}^{i \leq 6} w_i} \quad (4)$$

where w_i is the *weight* given to a face on the dice. The sum on the bottom ($\sum_{i=1}^{i \leq 6} w_i$) is the *normalization factor*. It describes the total the likelihood of a roll giving all faces, allowing us to determine the relative probability of each specific face.

If a dice is fair, all faces have the same weight:

$$p_{\text{Ⓢ}} = \frac{1}{1 + 1 + 1 + 1 + 1 + 1} = \frac{1}{6} \approx 0.167$$

Note we could assign any weight we wanted, as long as it was the same for all faces:

$$p_{\text{Ⓢ}} = \frac{0.001}{0.001 + 0.001 + 0.001 + 0.001 + 0.001 + 0.001} = \frac{0.001}{0.006} \approx 0.167$$

If we want to bias our results to get more Ⓢ , we could put more weight on that face. What if put a weight of 10 on the Ⓢ face, while setting the weight on every other face to 1?

$$p_{\text{Ⓢ}} = \frac{10}{1 + 1 + 1 + 1 + 1 + 10} = \frac{10}{15} \approx 0.667$$

We could even imagine an exotic dice where every face had a different weight. For example, imagine a dice weighted such that every face had the same weight as its numerical value. In that case:

$$p_{\text{Ⓢ}} = \frac{6}{1 + 2 + 3 + 4 + 5 + 6} = \frac{6}{21} \approx 0.286$$

Finally, if we want to know the probability of a Ⓢ or a Ⓢ , we could write:

$$p_{\text{Ⓢ}} + p_{\text{Ⓢ}} = \frac{w_1}{\sum_{i=1}^{i \leq 6} w_i} + \frac{w_6}{\sum_{i=1}^{i \leq 6} w_i} = \frac{w_1 + w_6}{\sum_{i=1}^{i \leq 6} w_i}$$

We can summarize our brief tangent as follows:

1. *Weights* can be any (positive) number for each face.
2. To convert weight to probaility, we divide by sum of weights on all sides.
3. To get the probability of face a or face b , calculate $w_a + w_b$ over the sum of the weights on all sides.

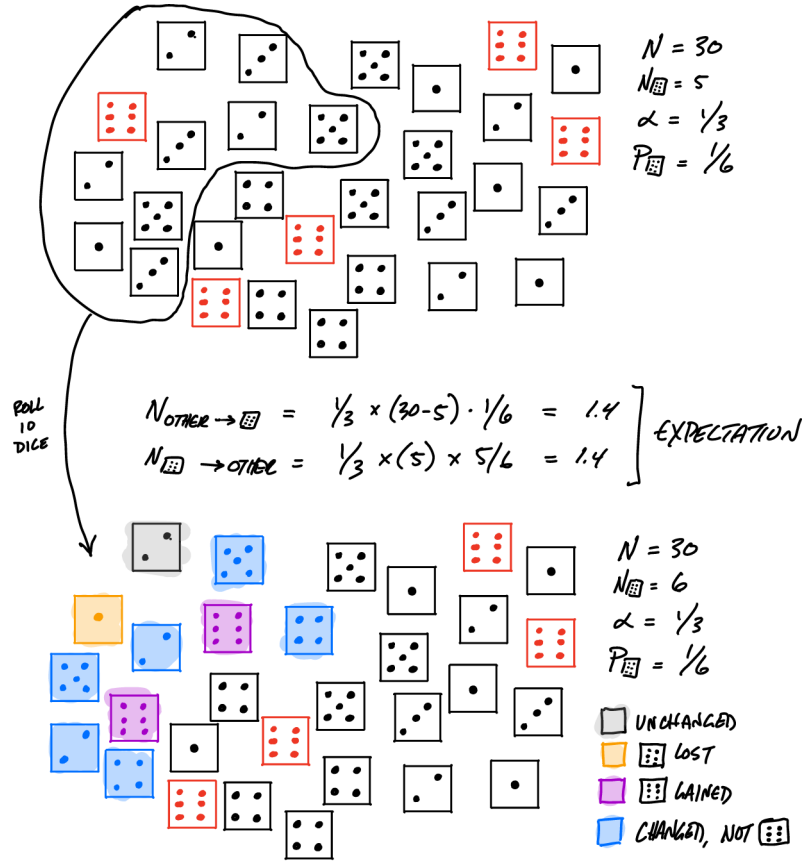


Figure 2: **The DFR in action.** We start with 30 dice, 5 of which are red . Our dice are fair ($p_{\text{red}} = 1/6$) and we flip $1/3$ of the dice. Because red are at a frequency of $5/30 = 1/6$ already, the number of red we expect to be gain matches the number we expect to lose. We then take a time step, selecting 10 dice and rolling them. In this example, one dice started red and remained red (gray); six dice started as something besides red and became something besides red (blue); one dice started red and became red (orange), and two dice started as something besides red and became red (purple). So, for this move, we gained two red and lost one red . Note that $\alpha = 1/3$ is too large to avoid double-rolls (see previous footnote); it is selected here for illustrative purposes, so we can see an appreciable number of dice rolls on one figure.

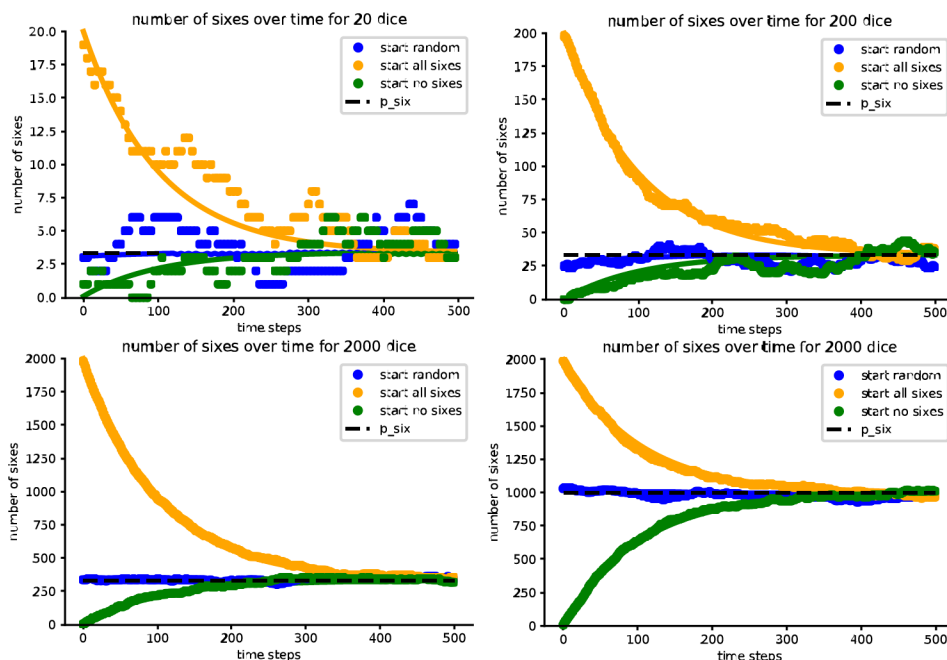


Figure 3: **DFR simulations converge to equilibrium.** Plots show the number of time steps on the x-axis, the number of [6] in the population on the y-axis. Points correspond to simulations starting at all [6] (orange), no [6] (green), or a random assortment of [1] , [2] , [3] , [4] , [5] , and [6] (blue). The first panel shows simulations with 20 dice, the second with 200 dice, the third with 2,000 dice, and the fourth with 2,000 dice that are weighted such that $p_{\text{[6]}} = 1/2$. The solid lines show the rate law we'll derive later.

2.4 Simulated reactions

Okay, now we have enough information about the DFR to do some simulations. We can then use these to develop a few ideas that apply to any chemical reaction. To do the simulations, we will create collections of computational dice and then randomly roll them according to the equations for $N_{\text{other} \rightarrow \text{[6]}}/\Delta t$, $N_{\text{[6]} \rightarrow \text{other}}/\Delta t$, and $p_{\text{[6]}}$ (Equations 2, 3, and 4). Fig 3 show the outcomes of several such simulations.

We can make a couple of observations from these simulations:

1. **Reactions become more predictable as the number of molecules increases.** As the number of dice increase, the curves become smoother. Hopefully, this is intuitive. Because dice rolling is a random process, it takes a large number of dice to average out all of the fluctuations. When we have only 20 dice, the fraction of [6] fluctuates up and down because even one low-probability roll has a huge effect on the outcome. When we have 2,000 dice, the fraction of [6] is robust to a few low probability events,

simply because there are so many dice that we average over. In chemical reactions, we usually have millions and millions of molecules, making the reaction dynamics quite predictably. (Intriguingly, this is *not* always true in a cell, where the number of reactants can sometimes involve only tens of molecules. A cell might have only ten copies of a transcription factor. This leads to *cellular noise*—a fascinating topic we will not cover, but that is worth reading about).

2. **Reactions converge to equilibrium whatever their starting conditions.** No matter how many A we start with, the simulations appear to converge on $p_{\text{A}}N$. If we pause for a moment, this is fairly remarkable. We have a “reaction” that has a direction, without a single interaction or bond. It even obeys Le Chatelier’s principle: if we add more reactants the reaction favors the product (green curve). If we make the reaction all product, some fraction of the molecules are eventually converted back to reactants (orange curve). Why does the reaction converge?

Observation #2 is particularly important. We will present two (complementary) views that describe why the reaction converges: kinetics and thermodynamics.

3 A kinetic explanation for convergence to equilibrium:

One way to think about why this reaction converges is to consider the rates of the forward and reverse reactions (Fig 4). Imagine we start with a collection of dice that are all A . At each step, we pick up a single dice and roll it. For the first step, we can only pick up A ; when we roll it, there is a 5/6 chance we end up with something besides A . This means that the most probable outcome of that first roll is to lower the number of A (Fig 4). For the first few rolls, we will still have more A than other numbers, so we will continue to favor rolls that lower the number of A . As we continue to roll dice, however, the number of non- A dice will increase, eventually allowing transitions from non- A back to A . Finally, after enough time, the rate of A loss and A gain offset offset, leading to the equilibrium value of N_{A} .

Put another way, at the beginning of the reaction, the high concentration of A means we will favor reactions where A is the reactant and where other states of the dice are the product. This bias away from A decays as the concentration of A goes down and the concentrations of other states of the dice go up.

3.1 Derive a rate law

So, what is the final value for N_{A} we expect to observe? To ask this question, we can derive a “rate law” that describes the reaction kinetics at play.³

³Calculus (particularly differential equations) is intimidating for some students. Even if you can’t do the steps below yourself, hopefully you can understand the steps we take in this derivation.

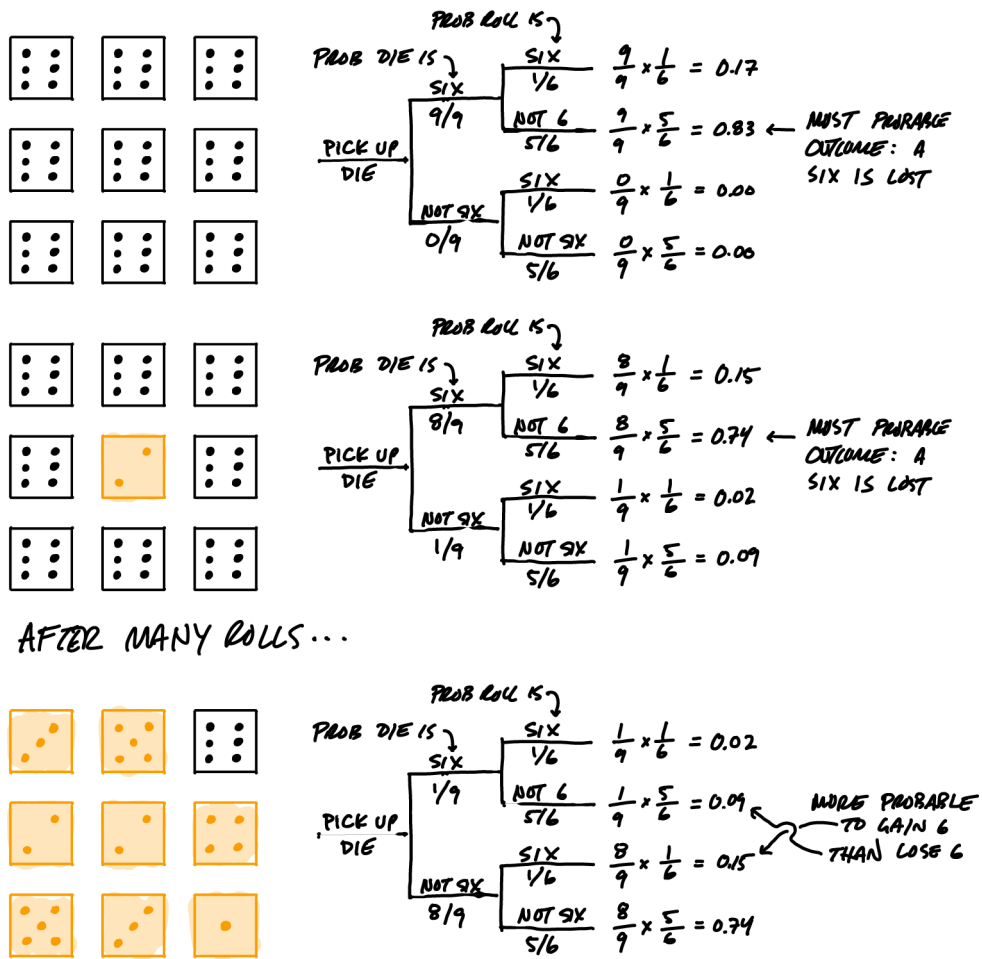


Figure 4: When there are excess sixes, rolls favor loss of sixes. Plot shows three time steps of the DFR. The sets of dice on the left show the current state of the dice collection. The tree diagrams to the right show the relative probability for all outcomes of a roll of one dice given the configuration of the dice to the left.

We can start by writing out a differential equation: the change in the number of \mathbb{I} is given by how many appear at each time step, less the number that are lost.

$$\frac{dN_{\text{six}}}{dt} = \frac{N_{\text{other} \rightarrow \mathbb{I}}}{dt} - \frac{N_{\mathbb{I} \rightarrow \text{other}}}{dt}$$

Substitute equations 2 and 3 above and simplify:

$$\frac{dN_{\mathbb{I}}}{dt} = \alpha \cdot (N - N_{\mathbb{I}}) \cdot p_{\mathbb{I}} - \alpha \cdot N_{\mathbb{I}} \cdot (1 - p_{\mathbb{I}})$$

$$\frac{dN_{\mathbb{I}}}{dt} = \alpha (p_{\mathbb{I}}N - p_{\mathbb{I}}N_{\mathbb{I}} - N_{\mathbb{I}} + p_{\mathbb{I}}N_{\mathbb{I}})$$

$$\frac{dN_{\mathbb{I}}}{dt} = \alpha (p_{\mathbb{I}}N - N_{\mathbb{I}})$$

Rearrange so the $dN_{\mathbb{I}}$ and dt terms are on opposite sides of the equation:

$$dN_{\mathbb{I}} = \alpha (p_{\mathbb{I}}N - N_{\mathbb{I}}) dt$$

$$\frac{1}{p_{\mathbb{I}}N - N_{\mathbb{I}}} dN_{\mathbb{I}} = \alpha dt$$

Integrate both sides:

$$\int \frac{1}{p_{\mathbb{I}}N - N_{\mathbb{I}}} dN_{\mathbb{I}} = \int \alpha dt$$

Yielding:

$$-\ln(p_{\mathbb{I}}N - N_{\mathbb{I}}) = \alpha t + C$$

To figure out C , plug in values we know. In this case, we know that at $t = 0$, we have some initial number of \mathbb{I} ($N_{\mathbb{I},0}$). (This is known as a boundary condition).

$$-\ln(p_{\mathbb{I}}N - N_{\mathbb{I},0}) = \alpha \cdot 0 + C$$

Once we know C , do some algebra to solve for $N_{\mathbb{I}}$:

$$-\ln(p_{\mathbb{I}}N - N_{\mathbb{I}}) = \alpha t - \ln(p_{\mathbb{I}}N - N_{\mathbb{I},0})$$

$$-\ln(p_{\mathbb{I}}N - N_{\mathbb{I}}) + \ln(p_{\mathbb{I}}N - N_{\mathbb{I},0}) = \alpha t$$

Use the rule $\log(a) - \log(b) = \log(a/b)$:

$$-\ln\left(\frac{p_{\mathbb{I}}N - N_{\mathbb{I}}}{p_{\mathbb{I}}N - N_{\mathbb{I},0}}\right) = \alpha t$$

$$\begin{aligned}\frac{p_{\text{⚡}} N - N_{\text{⚡}}}{p_{\text{⚡}} N - N_{\text{⚡},0}} &= e^{-\alpha t} \\ p_{\text{⚡}} N - N_{\text{⚡}} &= (p_{\text{⚡}} N - N_{\text{⚡},0}) e^{-\alpha t} \\ N_{\text{⚡}} &= p_{\text{⚡}} N - (p_{\text{⚡}} N - N_{\text{⚡},0}) e^{-\alpha t}\end{aligned}$$

Simplify:

$$\begin{aligned}N_{\text{⚡}} &= p_{\text{⚡}} N - p_{\text{⚡}} N e^{-\alpha t} + N_{\text{⚡},0} e^{-\alpha t} \\ N_{\text{⚡}} &= p_{\text{⚡}} N (1 - e^{-\alpha t}) + N_{\text{⚡},0} e^{-\alpha t}\end{aligned}\tag{5}$$

This rate law is shown as the solid lines in Fig 3. Happily, the simulated results and the analytical result overlay completely. This suggests that we did not make a mistake in either our computer simulation or derivation above.⁴

Now that we have a rate law, we can ask about the equilibrium state of the reaction. What happens as $t \rightarrow \infty$? If we substitute in ∞ , we find:

$$\begin{aligned}N_{\text{⚡}} &= p_{\text{⚡}} N (1 - e^{-\infty}) + N_{\text{⚡},0} e^{-\infty} \\ N_{\text{⚡}} &= p_{\text{⚡}} N\end{aligned}$$

After infinite time, we've the number of ⚡ is given by the probability of ⚡ times the total number of dice. When our dice reach this special concentration, $p_{\text{⚡}} N$, the rate of ⚡ formation precisely offsets the rate of ⚡, leading to this equilibrium value.

3.2 Link to biochemistry

So how do these models for dice relate to real chemistry? In a chemistry text book, you'll find that a first-order rate constant looks something like this:

$$\frac{d[\text{product}]}{dt} = k \cdot [\text{reactant}]$$

where k is a *rate constant*, $[\text{product}]$ is the concentration of product and $[\text{reactant}]$ is the concentration of reactant. It turns out, we already wrote discrete versions of this:

$$\frac{N_{\text{other} \rightarrow \text{⚡}}}{\Delta t} = \alpha \cdot (N - N_{\text{⚡}}) \cdot p_{\text{⚡}}.$$

In our discrete system with unimolecular reactions, $N - N_{\text{⚡}}$ is operating like a concentration ("how many non-⚡ are available to react?"). This means that the rate constant for the reaction is:

$$k_{\text{other} \rightarrow \text{⚡}} = \alpha \cdot p_{\text{⚡}}.$$

Our rate constant has two basic chunks: how many tries you make per turn (remember, α is the fraction of the dice we pick up and roll on a turn) and the

⁴Or at least that we made the same mistake in both places!

probability a reaction occurs per try (p_{II}). We will revisit it when we discuss the *Arrhenius equation*.⁵

How about equilibrium constants? Can we understand what they mean in terms of the dice reaction? You have probably seen that, for a two-state reaction, you can write:

$$K_{eq} = \frac{k_{forward}}{k_{reverse}}$$

We already have $k_{forward}$ from above ($k_{other \rightarrow \text{II}}$). By analogy:

$$k_{reverse} = k_{\text{II} \rightarrow other} = \alpha \cdot (1 - p_{\text{II}}).$$

If we substitute this into the expression for K_{eq} above, we find that

$$\frac{k_{forward}}{k_{reverse}} = \frac{\alpha p_{\text{II}}}{\alpha(1 - p_{\text{II}})} = \frac{p_{\text{II}}}{1 - p_{\text{II}}}.$$

You have also probably seen that:

$$K_{eq} = \frac{[products]}{[reactants]}.$$

Can we get there with the DFR? From our rate law, we found that the final concentration of II was $p_{\text{II}}N$. If we substitute this into our equation for K_{eq} above, we get:

$$K_{eq} = \frac{N_{\text{II}}}{N - N_{\text{II}}} = \frac{p_{\text{II}}N}{N - p_{\text{II}}N} = \frac{p_{\text{II}}}{1 - p_{\text{II}}}.$$

Hopefully from this quick analysis you can see better what first-order rate constants and equilibrium constants mean.

- k (first-order rate constant): How often one tries a reaction times the probability the reaction occurs.
- $K_{eq} = \frac{k_{forward}}{k_{reverse}} = \frac{[products]}{[reactants]}$: The concentration of products and reactants that, when combined with the rates of the forward and reverse reaction, cause the two reactions to exactly offset one another. At $N_{\text{II}} = p_{\text{II}}N$, the rate of losing II is exactly offset by the rate of gaining II and the reaction has equilibrated.

⁵To whet your appetite, you may have seen:

$$k = A \cdot e^{-E^\ddagger/RT}.$$

A is a prefactor that controls how often a reaction samples (“tries to cross”) an energy barrier. This is analogous to α . The other term, $\exp(-E^\ddagger/RT)$, is the probability the reaction crosses a barrier of height E^\ddagger at temperature T . This is analogous to p_{II} . This is a rough, as we are not discussing an energy barrier for our dice. But the basic formulation of “how often do you try?” times “how probable you are at succeeding?” occurs all over in chemical kinetics.

4 DFR Thermodynamics

A second way to understand the directionality of these reactions is by thinking about the relative probability of different arrangements of dice.

We'll start with a simple, preliminary argument. Imagine we start with a collection of random dice and start rolling. What's the probability we end up with all \boxplus at some point? Hopefully it is intuitive that there are many more ways to be something besides all \boxplus than to be all \boxplus . If we have two dice, there are 6 possibilities for the first dice, and 6 possibilities for the second dice. This means we have $6 \times 6 = 36$ different ways to arrange the dice. Since there is only one way to get $\boxplus\boxplus$, the probability of seeing $\boxplus\boxplus$ is $1/36 \approx 0.03$. The scaling relationship for the number of possible arrangements of the dice goes by $6^{N_{\text{dice}}}$. This means that even for four dice, there is only a $1/1,296$ chance of getting four \boxplus by chance. By the time we hit 100 dice, the odds of getting 100 \boxplus are absurdly low: 1.5×10^{-78} .

N_{dice}	possible states	number possible states	$p_{\text{all } \boxplus}$
1	$\boxminus, \boxplus, \boxtimes, \boxdiv, \boxdot, \boxplus$	$6^1 = 6$	$1/6 \approx 0.1667$
2	$\boxminus\boxminus, \boxminus\boxplus, \boxminus\boxtimes, \boxminus\boxdiv, \boxminus\boxdot, \boxminus\boxplus, \boxplus\boxminus, \boxplus\boxplus, \dots, \boxplus\boxplus$	$6^2 = 36$	$1/36 \approx 0.0278$
3	$\boxminus\boxminus\boxminus, \dots, \boxplus\boxplus\boxplus$	$6^3 = 216$	$1/216 \approx 0.0046$
4	$\boxminus\boxminus\boxminus\boxminus, \dots, \boxplus\boxplus\boxplus\boxplus$	$6^4 = 1296$	$1/1296 \approx 0.0008$
10	etc.	$6^{10} \approx 6.0 \times 10^7$	$\approx 1.7 \times 10^{-8}$
100	etc.	$6^{100} \approx 6.5 \times 10^{77}$	$\approx 1.5 \times 10^{-78}$

This, at its core, is the meaning of *entropy*. Systems end up in populating states with the most configurations associated with it. Since there are vastly more ways to end up as something besides all- \boxplus than all- \boxplus —just as there are vastly more ways to have a losing lottery number than a winning lottery number—it is essentially impossible

4.1 Equilibrium balances probable outcomes against outcomes that can happen in many ways

This argument lets us understand why we do not see something extreme like all \boxplus , but not why we end up at the equilibrium value of p_{\boxplus} . To do this, we need a general expression for calculating the probability of combinations of rolls. To get that, we're going to need to recall two basic rules from probability:

- **ANDs MULTIPLY.** If you have two independent events—like rolling \boxplus on one dice AND rolling \boxminus on a second dice—the probability of both events occurring is the *product* of the probabilities of each independent event ($p_{\boxplus} \times p_{\boxminus}$).
- **ORs ADD.** If you have two mutually exclusive events—like rolling a \boxplus OR rolling a \boxminus on the same dice—the probability of seeing one or the other is the *sum* of the two probabilities ($p_{\boxplus} + p_{\boxminus}$).

Let's develop our formula by asking what the probability of seeing exactly one 1 when rolling three dice is. We could roll 1 first and then roll something besides 1 twice. Since the dice are independent, the probability of this series of events is:

$$p_{11} \times (1 - p_{11}) \times (1 - p_{11}).$$

We could also roll something beside 1 first, roll 1 second, and then roll something beside 1 third:

$$(1 - p_{11}) \times p_{11} \times (1 - p_{11}).$$

Or, finally, we could roll something beside 1 twice and then roll 1 third:

$$(1 - p_{11}) \times (1 - p_{11}) \times p_{11}.$$

These series of events are mutually exclusive: if we only saw one 1 in three rolls, we either saw the 1 first, second, or third. The total probability of seeing one 1 is thus the sum of the probabilities of these exclusive series of events:

$$P_{one \times 1} = p_{11} \times (1 - p_{11}) \times (1 - p_{11}) + (1 - p_{11}) \times p_{11} \times (1 - p_{11}) + (1 - p_{11}) \times (1 - p_{11}) \times p_{11}.$$

We can factor and rearrange to yield:

$$P_{one \times 1} = 3 \times p_{11}^1 (1 - p_{11})^2$$

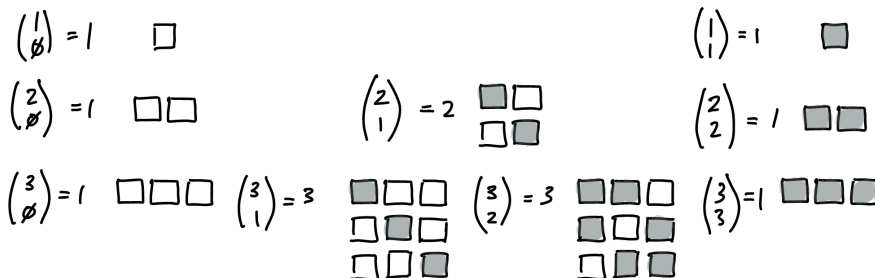
The “3” in this equation is called the *multiplicity*. It describes how many different ways we could achieve one 1—in this case, three. We raise p_{11} to the 1st power because we see it once, and $(1 - p_{11})$ to the 2nd power because we see it twice.

This calculation generalizes to:

$$P(n, k) = \binom{n}{k} p^k (1 - p)^{n-k} \quad (6)$$

where k is the number of 1 we observe out of n total dice that each have a probability p of coming up 1. $\binom{n}{k}$ is the *multiplicity* for a given roll (see box).

The notation $\binom{n}{k}$ (said “ n -choose- k ”) gives us the number possible ways that we can get k \blacksquare from n total dice. This is illustrated below for increasing n , where open squares are not \blacksquare and filled squares are \blacksquare .



The formula for $\binom{n}{k}$ is:

$$\binom{n}{k} = \frac{n!}{k!(n-k)!}.$$

As you might imagine, these numbers get very large as n increases, so it is often easier to estimate numerically rather than using this formula. The $\binom{n}{k}$ function is 'scipy.special.comb' in python, 'choose' in R, and 'COMBIN' in Excel.

Believe it or not, Equation 6 is a very powerful way to think about thermodynamics. **If you can understand how it works, you are on your way to understanding a ton of physical biochemistry.** One way to appreciate this is to plot the main parts of this equation as a function of increasing k —that is, going from no \blacksquare ($k = 0$) to all \blacksquare ($k = n$) (Fig 5).

First, consider the $\binom{n}{k}$ chunk (top row). This function is centered at 50%, meaning that the the highest number of configurations occurs for $k = n/2$. Hopefully this makes intuitive sense. (This can be seen graphically in the $n - \text{choose} - k$ box above: intermediate values of k maximize the number of ways to arrange the system.) Note also that the peak gets *large*. For the $n = 300$ case, there are 10^{88} possible arrangements of 150 \blacksquare and 150 not- \blacksquare dice.

Next, consider the $p^k(1-p)^{n-k}$ chunk (middle row). This function decreases exponentially from $k = 0$ to $k = n$ (note the log scale on the y-axis). This is because $(1-p) > p$ for a fair dice. A collection of rolls containing fewer \blacksquare will always be more probable than a collection of rolls with more \blacksquare . But, because this function goes as the $(n-k)$ th power, these numbers are even smaller than the $\binom{n}{k}$ values are large. For the $k = 150, n = 300$ case $\binom{n}{k} \approx 10^{88}$, but $p^k(1-p)^{n-k} \approx 10^{-129}$. Even the most likely set of rolls— $k = 0$, containing no \blacksquare —has a very low overall probability (1.8×10^{-24}).

If you combine $\binom{n}{k}$ with $p^k(1-p)^{n-k}$ (bottom) row, you end up with the total probability of observing $P(n, k)$. For clarity, we've put the numerical values of these functions in the table below for a few key values of k . In the table, one can see that the configuration with the most states from $\binom{n}{k}$ function ($k = 150, n = 300$) is obliterated by the tiny value $p^k(1-p)^{n-k}$ function. The

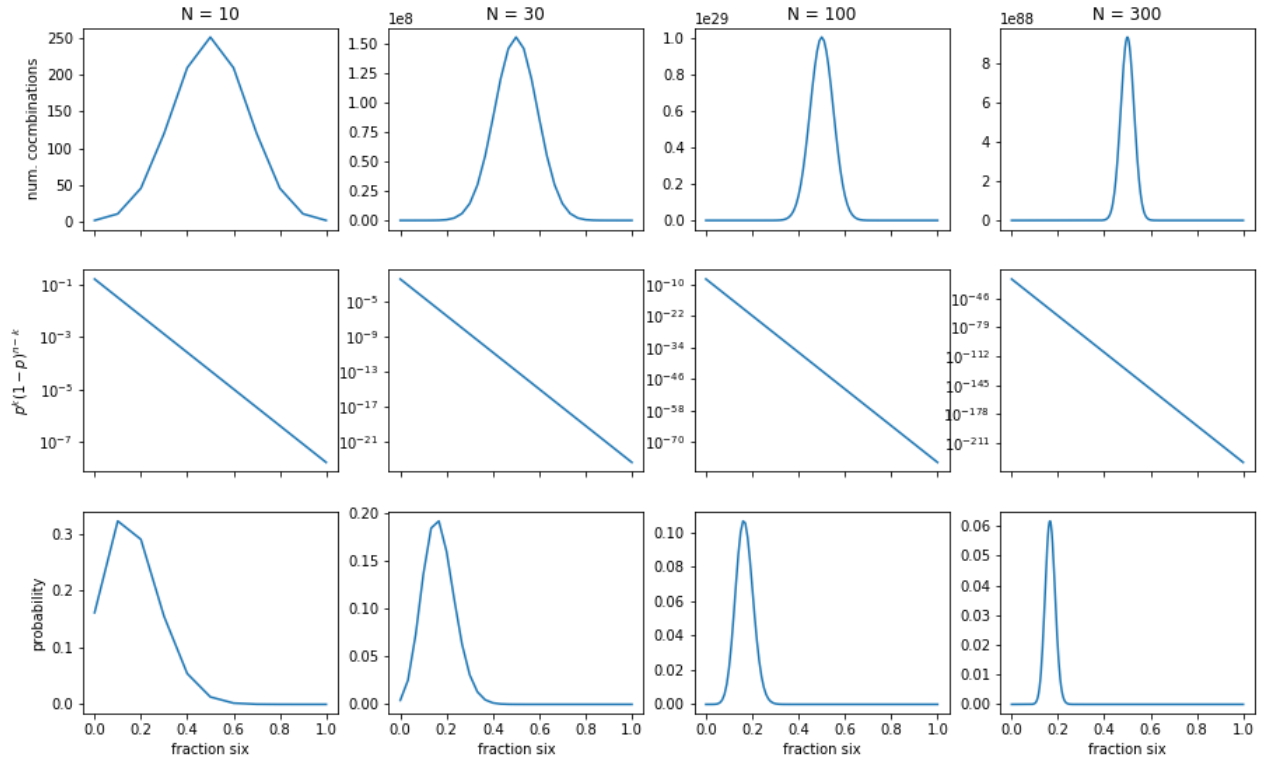


Figure 5: These panels show increasing total numbers of dice from left to right 10, 30, 100, and 300. The rows show $\binom{n}{k}$ (top), $p^k(1-p)^{n-k}$ (middle), and $P(n, k)$ (bottom). Note the log scale used for the $p^k(1-p)^{n-k}$ y-axis.

highest magnitude for $p^k(1-p)^{n-k}$ function ($k=0$), is still tiny. Since there is only one way to achieve this outcome—don't roll a single 1—it's overall probability remains very low. It's only at $k \approx 50$ that these two chunks come together to yield $P(n, k)$ with reasonable probabilities ($p = 0.06$).

n	k	$\binom{n}{k}$	$p^k(1-p)^{n-k}$	$P(n, k)$
300	0	1	1.8×10^{-24}	1.8×10^{-24}
300	50	3.1×10^{57}	2.0×10^{-59}	0.06
300	150	9.4×10^{88}	2.5×10^{-129}	2.4×10^{-40}
300	300	1	3.6×10^{-234}	3.6×10^{-234}

Put directly: the equilibrium value for the number of 1s is the result of a balance between 1) the probability of rolling a 1 versus something else ($p^k(1-p)^{n-k}$) and 2) tending towards states with maximum multiplicity ($\binom{n}{k}$).

4.2 Beginnings of a link to thermodynamics

Fine, you say: what does any of this have to do with thermodynamics? Let's make the connection explicit. Hopefully, in some chemistry class somewhere, you came across the following:

$$\Delta G = \Delta H - T\Delta S.$$

You likely remember that ΔG (the free energy) indicates whether a reaction is “spontaneous” or not. Maybe ΔH (the change in enthalpy) was described in terms of the difference in the energies of bonds formed in the products and reactants. Finally, ΔS (the change in entropy) might have been explained as a change in the amount of disorder between the products and reactants. Believe it or not, analogs of these exist even in something as simple as the DFR.

To show this, let's calculate the relative probability of being in the $k = k_2$ state versus the $k = k_1$ state. The ratio of these two probabilities is:

$$\frac{P(n, k_2)}{P(n, k_1)} = \frac{\binom{n}{k_2} p^{k_2} (1-p)^{n-k_2}}{\binom{n}{k_1} p^{k_1} (1-p)^{n-k_1}}$$

The $\binom{n}{k_1}$ notation is a bit unwieldy, so let's replace it with a W_k , which will still represent the number of arrangements that are possible for k 1s:

$$W_k = \binom{n}{k}.$$

$$\frac{P(n, k_2)}{P(n, k_1)} = \frac{W_{k_2} p^{k_2} (1-p)^{n-k_2}}{W_{k_1} p^{k_1} (1-p)^{n-k_1}}$$

To make things simpler, let's take the $-\ln$ of both sides. (This makes it way easier to deal with a probability like 10^{-234} because: $-\ln(10^{-234}) = 234\ln(10) = 538.8$.)

$$-\ln \left[\frac{P(n, k_2)}{P(n, k_1)} \right] = -\ln \left[\frac{W_{k_2} p^{k_2} (1-p)^{n-k_2}}{W_{k_1} p^{k_1} (1-p)^{n-k_1}} \right]$$

Because $\log(a \cdot b) = \log(a) + \log(b)$:

$$-\ln \left[\frac{P(n, k_2)}{P(n, k_1)} \right] = -\ln \left[\frac{W_{k_2}}{W_{k_1}} \right] - \ln \left[\frac{p^{k_2} (1-p)^{n-k_2}}{p^{k_1} (1-p)^{n-k_1}} \right]$$

Hopefully this beginning to look an awful lot like things like G , H , and S . As formulated by chemists,

$$\Delta G = -RT \ln(K_{eq}) = -RT \ln \left(\frac{[product]}{[reactant]} \right)$$

This is directly analogous to:

$$-\ln \left[\frac{P(n, k_2)}{P(n, k_1)} \right]$$

Just like ΔG , this measures which dice configuration is more probable at equilibrium, with a negative value indicating that k_2 is more favorable than k_1 . If we put in $k_1 = 0$ and $k_2 = 50$ (meaning we go from no 1's to 1/6 1's), this gives us

$$-\ln \left[\frac{P(300, 50)}{P(300, 0)} \right] \approx -33.7$$

This means that if we started at $k = 0$, the system would *spontaneously* go to $k = 50$. This is exactly the meaning for ΔG usually given in a chemistry textbook.

This probability is determined by two, offsetting terms. The first measures the difference in the number of possible arrangements of dice between the k_1 and k_2 . This is also highly favorable. This is because there are many more ways to arrange 50 1's than 0 1's.

$$-\ln \left[\frac{W_{k_2}}{W_{k_1}} \right] = -\ln \left[\frac{W_{50}}{W_0} \right] \approx -132.4$$

This is a change in *entropy*: the system tending towards states with higher multiplicity.

Finally, this tendency towards states of higher multiplicity is opposed by the fact that increasing the number of 1's means having less likely rolls: more 1/6 1's and fewer 5/6 not-1's. This is captured in the second term, which quantifies the probability of the individual "microstates" (rolls) that make up each "state" (1's or not-1's).

$$-\ln \left[\frac{p^{k_2} (1-p)^{n-k_2}}{p^{k_1} (1-p)^{n-k_1}} \right] = -\ln \left[\frac{p^{50} (1-p)^{300-50}}{p^{50} (1-p)^{300-0}} \right] \approx 98.7$$

This term is positive and *opposes* the entropic drive towards more states. This is behaving similarly to an *enthalpy* in this case, where the energy stored in bonds can become less favorable in a reaction if there if this is offset by a change in entropy.

Key points:

1. The probability of seeing exactly k H out of n rolls, where the probability of rolling H is:

$$P(n, k) = \binom{n}{k} p^k (1 - p)^{n-k}$$

2. The chunks of this equation correspond to the typical breakdown of ΔG , ΔH , and ΔS in chemical reactions.
 - (a) $P(n, k)$ measures the relative probability of each state – the free energy
 - (b) $\binom{n}{k}$ describes the multiplicity of each state — the entropy
 - (c) $p^k (1 - p)^{n-k}$ measures the probability of the rolls required to make the state – the enthalpy.