BE/Bi 25: Biophysical Chemistry

Justin Bois

Caltech

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1 Why study biophysical chemistry?

This course is entitled *Biophysical Chemistry*. The subject of biophysical chemistry has several meanings, depending on whom you are asking. A course on this topic at one university may look quite different from one at another.

So, what is biophysical chemistry within the context of this course? We will look at what it *could* be, and then discuss what our main goals are.

1.1 It could be a theoretical topic list

Biophysical chemistry is often split into two pieces, study of dynamics and study of equilibria. The former is referred to as **chemical kinetics** and the latter as **thermodynamics**. Strangely, the word "dynamics" is part of thermo*dynamics*, but thermodynamics is not really a study of how systems change over time, but rather how they behave at equilibrium, which is usually their steady-state, static behavior.[1] The topics do have contact points where they are related, but by and large can be studied separately.

Common topics in thermodynamic study of biochemical systems include

- Statistical mechanics,
- Thermodynamic potentials,
- Equilibrium conditions,
- Solution thermodynamics,
- Stability and phase separation,
- Electrochemistry.

In the study of chemical kinetics, the following topics are often included.

- Mass action
- Determination of rate constants (activation energy, transition state theory)
- Thermal diffusion
- Mathematical solution of rate equations
- Enzyme kinetics

There are of course more, but these topics are common and in many ways foundational.

[1] The etymology of the term is not even clear!

1.2 It could be a list of techniques

So much of physical biochemistry involves *measuring* properties related to the above topics. As the list of topics falling under the umbrella of biophysical chemistry is long, so is the list of measurement techniques. Some of the common ones covered in courses like this one are

- Stop flow reactors,
- Continuous flow reactors,
- Batch reactors,
- Absorbance,
- Fluorescence,
- Scattering,
- Fluorescence anisotropy,
- Fluorescence resonance energy transfer,
- Sedimentation,
- Differential scanning calorimetry,
- Isothermal titration calorimetry,
- Equilibrium dialysis,
- Surface plasmon resonance,
- Voltammetry,
- Many more!

Through studying these techniques, the theoretical underpinnings must also be mastered, and many courses are structured around techniques.

1.3 It could just be structural biology

For many instructors, biophysical chemistry is all about structural biology. Topics include

- Secondary structure,
- Tertiary structure,
- Structural domains and motifs,
- Structural dynamics,

- Electrostatics,
- Polar/hydrophobic interactions.

There are also myriad structural techniques, including

- X-ray crystallography,
- Electron microscopy,
- Nuclear magnetic resonance,
- Mass spectrometry,
- Many more!

1.4 It could be foundational for understanding life

In his book, *What is Life?: Five Great Ideas in Biology*, Paul Nurse writes, "Most aspects of life can be understood rather well in terms of physics and chemistry, albeit an extraordinary form of chemistry that is highly organized, and of sophistication that cannot be matched by any inanimate process." He goes on to say, "Ultimately, life emerges from the relatively simple and well-understood rules of chemical attraction and repulsion, and the making and breaking of chemical bonds." From this perspective, a clear understanding of biophysical chemistry is essential to understand the living world.

1.5 It could be foundational for engineering life

Bioengineers use the molecules of the living world as building materials for the systems they engineer. As is the case with steel or concrete, the engineer needs to know their building materials to use them effectively. Some courses, such as those that use the book by Wittrup, Tidor, Hackel, and Sarkar (one of the textbooks for this course), take an engineering approach to biophysical chemistry, with an eye toward *using* biomolecules as engineering material.

1.6 So, what is biophysical chemistry for this course?

In this course, we will focus both on chemical kinetics and thermodynamics with an eye toward engineering (though that is not our only goal). While these lecture notes will provide most of the material, they are based upon and complement the books by Wittrup, Tidor, Hackel and Sarkar (*Quantitative Fundamentals of Molecular and Cellular Bioengineering*) and Kuriyan, Konforti, and Wemmer (*The Molecules of*

Life: Physical and Chemical Principles). We will introduce measurement techniques as we go along, but they will not be the main focus of the course. We will not delve too much into structural biology. (Caltech has two or three courses on structural biology offered in the Biochemistry and Molecular Biophysics Option.)

Traditionally in chemical and bioengineering curricula, thermodynamics is taught before kinetics. Indeed in Kuriyan, Konforti, and Wemmer's book, thermodynamics is also presented first. In my experience, and in those shared by students I know, thermodynamics tends to be a conceptually more difficult subject than chemical kinetics. Of course, as you dive deeper into the respective subjects, you will find richness and subtlety, and both subjects will present difficult problems and opportunities for hard thinking. But for an early foray into these subjects, as we are embarking on here, I think that chemical kinetics is a less intimidating subject and a natural one to begin with.

So, we will spend the first half of the course learning about chemical kinetics, mostly from the perspective of mass action kinetics, with special focus on enzyme catalyzed reaction dynamics. In the second half of the course, we will introduce thermodynamics starting with a statistical description of entropy and building out the thermodynamic potentials from there. We will then explore classic topics of foundational importance such as solution thermodynamics and equilibrium conditions.

The hope is that at the end of the course, you will be equipped to proceed with your training as a bioengineering. Indeed, almost *all* of the courses in the Caltech Bioengineering curriculum build off of the topics presented in this course.

Part I

Kinetics

2 Mass action kinetics

In this lesson, we will explore concepts behind mass action kinetics through a series of examples and intuitive reasoning.

We will first reason the main idea behind mass action kinetics, that the rate at which a chemical reaction proceeds is proportional to the product of the concentrations of its reactants. We will then discuss some techniques for solving the resulting differential equations describing the dynamics of concentrations of chemical species.

2.1 A simple illustrative example: Exponential decay

Let us first dive into chemical kinetics with a simple example. Imagine we have a small test tube that has a solution of RNA oligomers, all with the same sequence, in it. This is something we could buy, for example, from Integrated DNA Technologies. These are usually kept frozen, but we are going to just let this solution sit out on the bench top at room temperature. Even in the absence of ribonucleases that we are constantly shedding and can rapidly degrade RNA, RNA degrades rather quickly. Of course the rate of degradation is highly dependent on buffer conditions, but as a rough estimate, the half life of RNA at room temperature is on the hours-to-days time scale. So we ask the question, what is the concentration of RNA in the test tube over time? More specifically, if we define by c to be the concentration of RNA in the test tube, we would like to write an expression for $c(t)$, taking $c(0) = c_0$. For our sake of discussion here, let us say that $c_0 = 10$ nM.

Let us try to reason what $c(t)$ may be by performing a thought experiment. We do not really know the process by which the RNA may degrade, but we can assume a very simple process in which within a small window of time of length Δt an RNA molecule has some probability of degrading. Let's say that probability is θ. So, the probability that an RNA molecule does *not* decay in a small time window of length Δt is $1 - \theta$. Let us now consider a single RNA molecule. At time zero, it has not decayed. At time Δt, it has a probability of $1 - \theta$ of still being around. At time $2\,\Delta t$, it has a probability of $(1 - \theta)^2$ of still being around, since it had a probability of $1 - \theta$ of *not* decaying in the first time window and a probability of $1 - \theta$ of not decaying in the second time window. Note here that we are assuming each time window is independent. So, in time $t = n\,\Delta t$, the probability that the RNA molecule will still be around is $(1 - \theta)^n$. We can write the probability that a given molecule has not decayed as

$$P_{\text{not decayed}}(t) = (1 - \theta)^n = (1 - \theta)^{t/\Delta t} = \left((1 - \theta)^{1/\Delta t}\right)^t, \tag{2.1}$$

since $t = n\,\Delta t$. Now, if we define

$$k = -\frac{1}{\Delta t}\,\ln(1-\theta), \tag{2.2}$$

which is strictly nonnegative, since $0 \leq \theta \leq 1$, so that

$$(1-\theta)^{1/\Delta t} = \mathrm{e}^{-k}, \tag{2.3}$$

we have

$$P_{\text{not decayed}}(t) = \mathrm{e}^{-kt}. \tag{2.4}$$

So, the probability of being around decays exponentially over time.[2]

If we assume each RNA molecule in our test tube is independent and has the same kind of decay process, we can write that the *number* of non-decayed RNA molecules is

$$N_{\text{not decayed}}(t) = N_0\,P_{\text{not decayed}}(t) = N_0\,\mathrm{e}^{-kt} = c_0 V\,\mathrm{e}^{-kt}, \tag{2.5}$$

where N_0 is the initial number of RNA molecules, which is given by $N_0 = c_0 V$, where V is the volume of our little test tube. To get the concentration, we have $c(t) = N_{\text{not decayed}}(t)/V$, or

$$c(t) = c_0\,\mathrm{e}^{-kt}. \tag{2.6}$$

With this solution, we can make a plot of the concentration of RNA over time, assuming we start with $c_0 = 10$ nM. If the half life is about 10 hours, we can estimate k.

$$\frac{c(t_{1/2})}{c_0} = \frac{1}{2} = \frac{c_0\,\mathrm{e}^{-kt_{1/2}}}{c_0} = \mathrm{e}^{-kt_{1/2}} \;\Rightarrow k = \frac{\ln 2}{t_{1/2}} \approx 0.07\ \mathrm{hr}^{-1}. \tag{2.7}$$

We could use a computer to generate a plot for this, but it often helps intuition to *sketch* a plot. Such a sketch is shown in Fig. 1. When making sketches of exponential decays, I use the rule of thumb that the level decays to about a third of its starting level in time $1/k$, in this case about 14 hours.

While this is a pleasing result that followed from our thought experiment and applying a bit of probability, as will become clear as we work through this lesson, we almost always first write expressions for concentrations over time as *differential* equations. Differentiating the above expression with respect to time, we have

$$\frac{\mathrm{d}c}{\mathrm{d}t} = -k\,c_0\,\mathrm{e}^{-kt} = -kc. \tag{2.8}$$

[2] I have been a bit cavalier here. To be more formal, you can read about how an Exponential distribution is a continuous analog of a Geometric distribution.

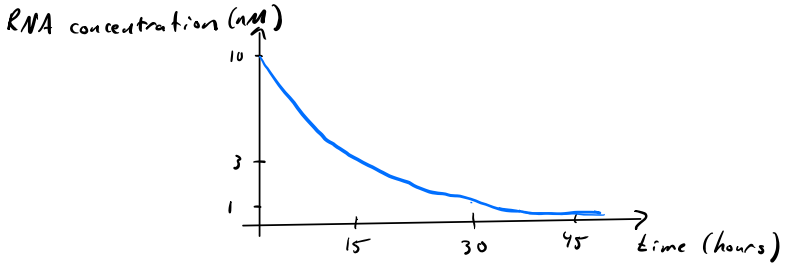


Figure 1: Time course of degradation of RNA in a test tube.

This is an interesting result. The *rate* of decay (which is $-\mathrm{d}c/\mathrm{d}t$) of the RNA is directly proportional to the concentration of the RNA. This makes sense if we do another little thought experiment. Say we have two test tubes, one with twice as concentrated RNA as the other. Then, at any small time window, there are twice as many decay events in the more concentrated tube than the other. So the *change* in concentration per unit time (that is the time derivative of the concentration) should be proportional to the concentration of the species undergoing the decay.

As we will further explore, if we consider a chemical reaction, **mass action kinetics** dictates that the rate at which a reaction proceeds is proportional to the product of the concentrations of its reactants. For our present example of decaying RNA molecules, the reaction is RNA $\longrightarrow \emptyset$. We defined c as the concentration of RNA, so the rate of reaction, in this case decay, is proportional to c, or

$$\text{rate of reaction} = kc, \tag{2.9}$$

where k is the constant of proportionality, referred to as a **rate constant**. Since RNA is degraded, the rate of reaction is given by the negative time derivative of the RNA concentration, or

$$\text{rate of reaction} = -\frac{\mathrm{d}c}{\mathrm{d}t} = kc. \tag{2.10}$$

2.2 Another illustrative example: dimer formation

Let us now consider another process, that of dimer formation. Say we have a protein A that forms dimers at a certain pH, but is monomeric at a different pH. We start off with lots of monomeric A around and then suddenly change the pH so that dimers can form. The chemical reaction here is

$$\mathrm{A} + \mathrm{A} \longrightarrow \mathrm{AA}. \tag{2.11}$$

We can again do a thought experiment here. Let's say that if two A molecules are adjacent to each other, they may undergo a dimerization reaction with probability θ

in some small time window. To consider adjacency, let us divide up the space in our test tube into N little boxes, and each box may contain an A monomer, an AA dimer, or solvent. We will consider one box in particular, which we will call "Our Box." The probability that an A monomer is in Our Box at a given time is N_A/N, where N_A is the number of A monomers in the test tube. In three dimensions, there are six adjacent boxes to Our Box, so the probability than an A monomer is in at least one of those boxes is one minus the probability that *no* A monomers are adjacent, or

$$1 - \left(\frac{N_\mathrm{s} + N_\mathrm{AA}}{N}\right)^6, \tag{2.12}$$

where N_s is the number of boxes containing solvent, such that $N_\mathrm{s} + N_\mathrm{A} + N_\mathrm{AA} = N$. Using this relation, we can rewrite the probability that an A monomer is in at least one of the boxes as

$$1 - \left(\frac{N_\mathrm{s} + N_\mathrm{AA}}{N}\right)^6 = 1 - \left(1 + \frac{N_\mathrm{A}}{N_\mathrm{s} + N_\mathrm{AA}}\right)^{-6}. \tag{2.13}$$

Thus, the probability of a reaction happening at Our Box in some small time window Δt is

$$\theta \, \frac{N_\mathrm{A}}{N} \left(1 - \left(1 + \frac{N_\mathrm{A}}{N_\mathrm{s} + N_\mathrm{AA}}\right)^{-6}\right). \tag{2.14}$$

We can approximate and simplify this expression by noting that typically $N_\mathrm{s} \gg N_\mathrm{A}, N_\mathrm{AA}$, such that $N \approx N_\mathrm{s}$ and $N_\mathrm{s} + N_\mathrm{AA} \approx N$, giving

$$\text{prob. of rxn in Our Box in } \Delta t \approx \theta \, \frac{N_\mathrm{A}}{N} \left(1 - \left(1 + \frac{N_\mathrm{A}}{N}\right)^{-6}\right) \tag{2.15}$$

Further, because $N_\mathrm{A}/N \ll 1$, we can perform the Taylor expansion

$$\left(1 + \frac{N_\mathrm{A}}{N}\right)^{-6} = 1 - \frac{6N_\mathrm{A}}{N} + \mathcal{O}\left(\left(\frac{N_\mathrm{A}}{N}\right)^2\right) \tag{2.16}$$

to get

$$\text{prob. of rxn in Our Box in } \Delta t \approx 6\theta \left(\frac{N_\mathrm{A}}{N}\right)^2. \tag{2.17}$$

All of the other boxes in the test tube are the same as Our Box, and if we assume they are all independent, then the probability of having a dimerization reaction in some small time window is

$$\text{prob. of rxn in } \Delta t \approx 6N\theta \left(\frac{N_\mathrm{A}}{N}\right)^2. \tag{2.18}$$

This means that the *rate* of production of dimers (considering Δt) is proportional to this expression, or

$$\text{rate of dimer production} = \frac{\mathrm{d}N_{\mathrm{AA}}}{\mathrm{d}t} \propto \frac{N_{\mathrm{A}}^2}{N}. \tag{2.19}$$

Remember that we divided the volume of the test tube up into boxes. The total number of boxes is proportional to the volume V. N is proportional to V, which means that

$$\frac{\mathrm{d}N_{\mathrm{AA}}}{\mathrm{d}t} \propto \frac{N_{\mathrm{A}}^2}{V}. \tag{2.20}$$

The *concentration* of dimers is N_{AA}/V. Substituting this gives

$$V\frac{\mathrm{d}c_{\mathrm{AA}}}{\mathrm{d}t} \propto \frac{N_{\mathrm{A}}^2}{V}. \tag{2.21}$$

Defining the constant of proportionality as the rate constant k, and noting that the concentration of monomer is $c_{\mathrm{A}} = N_{\mathrm{A}}/V$, we have

$$\frac{\mathrm{d}c_{\mathrm{AA}}}{\mathrm{d}t} = k\,c_{\mathrm{A}}^2. \tag{2.22}$$

This is again the result we stated earlier as a rule for mass action kinetics; the rate of a reaction is proportional to the product of the concentration of its reactants. Even though both reactants in this case are A, it takes two of them to make a dimer, so we have to square the monomer concentration in the rate expression.

2.3 Mass is conserved in chemical reactions

In our example of degradation of RNA, we wrote the reaction as $\mathrm{RNA} \longrightarrow \emptyset$. Really, we should have written $\mathrm{RNA} \longrightarrow \text{degraded RNA}$. The mass of the RNA did not go anywhere. We could write an expression for the rate of production of degraded RNA as

$$\frac{\mathrm{d}c_{\text{degraded RNA}}}{\mathrm{d}t} = -\frac{\mathrm{d}c}{\mathrm{d}t}, \tag{2.23}$$

because for every RNA molecule that is degraded, a degraded RNA molecule is formed.[3] The total mass of RNA/degraded RNA is therefore conserved.

$$\frac{\mathrm{d}c_{\text{total RNA}}}{\mathrm{d}t} = \frac{\mathrm{d}c_{\text{degraded RNA}}}{\mathrm{d}t} + \frac{\mathrm{d}c}{\mathrm{d}t} = -\frac{\mathrm{d}c}{\mathrm{d}t} + \frac{\mathrm{d}c}{\mathrm{d}t} = 0. \tag{2.24}$$

[3] Of course, upon degradation, many pieces of degraded RNA are formed. Here, we are defining $c_{\text{degraded RNA}}$ to be the concentration of a molecule formed from taping together all of the pieces of a singled degraded RNA molecule.

Similarly, in the dimerization example, total mass of A must be conserved. We need to bear in mind that two monomers are consumed for every dimer that is produced, such that

$$\frac{\mathrm{d}c_\mathrm{A}}{\mathrm{d}t} = -2\,\frac{\mathrm{d}c_\mathrm{AA}}{\mathrm{d}t} = -2k\,c_\mathrm{A}^2, \tag{2.25}$$

so that

$$\frac{\mathrm{d}(\text{total concentration of A})}{\mathrm{d}t} = \frac{\mathrm{d}c_\mathrm{A}}{\mathrm{d}t} + 2\frac{\mathrm{d}c_\mathrm{AA}}{\mathrm{d}t} = 0. \tag{2.26}$$

Mass is in general conserved, and rate expressions must respect that.

2.4 The assumptions behind mass action kinetics

We went through two thought experiments to arrive at what I stated at the beginning of this lesson, that mass action kinetics dictates that the rate of a chemical reaction is proportional to the product of its reactants. It seems like a laborious route to a simple, intuitive principle. But going through those thought experiments made clear the assumptions and approximations that underlie use of mass action kinetics. Let us recap.

1. We assumed all reactions were independent of all others happening in the test tube.
2. We assumed there is no memory of past events; we considered only probability of reaction or collisions at a given moment.[4]
3. We assumed that the solutions were **dilute**. In the dimerization example, this was specifically that $N \gg N_\mathrm{A}, N_\mathrm{AA}$.

Taken together, these assumptions seem pretty restrictive. However, mass action kinetics are one of those theories that are *unreasonably effective*, meaning that mass action kinetics matches experiment way more often than we might think given these apparently restrictive conditions for which mass action kinetics applies.[5]

[4] Taken together, these two assumptions mean that we model chemical events as **Poisson processes**, a concept beyond the scope of this course.

[5] I think the Navier-Stokes equations are another example of unreasonably effective theory. They apply over a huge range of length scales and fluids can be treated as continua even when there are very few particles.

2.5 Mass action expressions for reversible reactions

In general, chemical reactions are **reversible**, meaning that if the reactants can be converted to products, so too can the products be converted to reactants. However, many chemical reaction are *effectively* irreversible, meaning that the reverse reaction is so slow that it does not really happen in practice. In the RNA degradation, it is *possible* that the pieces of a degraded RNA molecule may come back together to form an intact RNA molecules, but extremely unlikely, so the degradation reaction is effectively irreversible.

We can model reversible reactions in the same way we model irreversible ones when it comes to writing down rate expressions. Conceptually, we just need to treat the forward and reverse reactions as separate reactions.

As an illustrative example, consider again the dimerization reaction, except this time it is reversible,

$$\mathrm{A} + \mathrm{A} \underset{k_-}{\overset{k_+}{\rightleftharpoons}} \mathrm{AA}. \tag{2.27}$$

As is traditional, the arrows of chemical reactions are often annotated with an assigned chemical rate constant. This chemical reaction could instead be written as two chemical reactions.

$$\mathrm{A} + \mathrm{A} \xrightarrow{k_+} \mathrm{AA}, \tag{2.28}$$

$$\mathrm{AA} \xrightarrow{k_-} \mathrm{A} + \mathrm{A}. \tag{2.29}$$

According to mass action kinetics, the rate of the first reaction is $k_+ \, c_\mathrm{A}^2$, and the rate of the second reaction is $k_- \, c_\mathrm{AA}$.

This is fine and good for keeping track of the reactions and their rates, but we ultimately need to write down differential equations for the concentrations of the respective chemical species. In the forward reaction, monomeric A is consumed (two molecules per dimer created), and in the reverse reaction, monomeric A is produced (again, two molecules per dimer). Similarly, in the forward reaction, dimers are produced, while they are consumed in the reverse reaction. Properly doing the accounting, we can write down the differential equations.

$$\frac{\mathrm{d}c_\mathrm{A}}{\mathrm{d}t} = -2k_+ \, c_\mathrm{A}^2 + 2k_- \, c_\mathrm{AA}, \tag{2.30}$$

$$\frac{\mathrm{d}c_\mathrm{AA}}{\mathrm{d}t} = k_+ \, c_\mathrm{A}^2 - k_- \, c_\mathrm{AA}. \tag{2.31}$$

2.6 Stoichiometric coefficients

We have thus far expressed the reversible dimerization reaction in two ways. First, we write it as a single reversible reaction,

$$\mathrm{A} + \mathrm{A} \underset{k_-}{\overset{k_+}{\rightleftharpoons}} \mathrm{AA}. \tag{2.32}$$

Then, we separated the forward and reverse reactions.

$$2\,\mathrm{A} \xrightarrow{k_+} \mathrm{AA}, \tag{2.33}$$

$$\mathrm{AA} \xrightarrow{k_-} \mathrm{A} + \mathrm{A}. \tag{2.34}$$

We can alternatively write these two reactions in another way.

$$-2\,\mathrm{A} + \mathrm{AA} = 0, \quad \text{rate constant } k_+, \tag{2.35}$$

$$2\,\mathrm{A} - \mathrm{AA} = 0, \quad \text{rate constant } k_-. \tag{2.36}$$

The idea here is that the reactions are written as a sum to zero, where the coefficient in front of each species is its **stoichiometric coefficient**. In the first reaction, for example, two monomers are consumed (hence the -2 stoichiometric coefficient) and one dimer is created (hence the $+1$ stoichiometric coefficient). Similarly, in the second reaction, two monomers are created and one dimer is consumed. Comparing to the "arrow" way of writing reactions, as in equations (2.33) and (2.34), stoichiometric coefficients to the left of the arrow have negative signs and those to the right of the arrow have positive signs.

2.7 Prescription for mass action kinetics

More generally, a reaction involving species $\mathrm{S}_1, \mathrm{S}_2, \ldots, \mathrm{S}_{N_s}$ (where N_s here is the number of species and is not to be confused with the number of solvent molecules from earlier in this section) may be written may be written as

$$\sum_{j=1}^{N_s} \nu_j \mathrm{S}_j = 0, \tag{2.37}$$

where the ν_j are the stoichiometric coefficients. (If a chemical species does not appear in a reaction, its stoichiometric coefficient is zero.) If there is more than one reaction, we can write reaction i as

$$\sum_{j=1}^{N_s} \nu_{ij} \mathrm{S}_j = 0, \tag{2.38}$$

where ν_{ij} is the stoichiometric coefficient for species j in reaction i. For the reversible dimerization case we can take $S_1 = A$, $S_2 = AA$ and consider reaction 1 to be dimerization and reaction 2 to be dissociation. Then, $\nu_{11} = -2$, $\nu_{12} = 1$, $\nu_{21} = 2$ and $\nu_{22} = -1$.

Recall that mass action kinetics dictates that the rate of a reaction is proportional to the product of the concentrations of its reactants, and we call the constant of proportionality the chemical rate constant. Defining c_j to be the concentration of species S_j and k_i to be the chemical rate constant for reaction i, we can write an expression for the rate of reaction i as

$$\text{rate of reaction } i = k_i \prod_{j=1}^{N_s} c_j^{\phi_{ij}}, \tag{2.39}$$

where

$$\phi_{ij} = \begin{cases} |\nu_{ij}| & \text{if } \nu_{ij} < 0 \\ 0 & \text{otherwise.} \end{cases} \tag{2.40}$$

The parameter ϕ_{ij} is defined such that only reactants are included in the rate expression. This could equivalently be written as

$$\text{rate of reaction } i = k_i \prod_{j=1}^{N_s} c_j^{\left|\min(0,\nu_{ij})\right|}. \tag{2.41}$$

With this in hand, we can write the time derivative of c_j using mass action kinetics as

$$\frac{\mathrm{d}c_j}{\mathrm{d}t} = \sum_{i=1}^{N_r} \nu_{ij}(\text{rate of reaction } i) = \sum_{i=1}^{N_r} \nu_{ij}\, k_i \prod_{j=1}^{N_s} c_j^{\left|\min(\nu_{ij},0)\right|}, \tag{2.42}$$

where there are N_r total reactions.

This is all a bit formal. For clarity, let us consider an example. Consider a system with two reversible reactions,

$$\mathrm{AB} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{A} + \mathrm{B}, \tag{2.43}$$

$$\mathrm{AC} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \mathrm{A} + \mathrm{C}. \tag{2.44}$$

The differential equations describing the dynamics of the concentrations of the respective species are then

$$\frac{\mathrm{d}c_\mathrm{A}}{\mathrm{d}t} = k_1\, c_\mathrm{AB} - k_{-1}\, c_\mathrm{A}\, c_\mathrm{B} + k_2\, c_\mathrm{AC} - k_{-2}\, c_\mathrm{A}\, c_\mathrm{C}, \tag{2.45}$$

$$\frac{\mathrm{d}c_\mathrm{B}}{\mathrm{d}t} = k_1\, c_\mathrm{AB} - k_{-1}\, c_\mathrm{A}\, c_\mathrm{B}, \tag{2.46}$$

$$\frac{\mathrm{d}c_\mathrm{C}}{\mathrm{d}t} = k_2\, c_\mathrm{AC} - k_{-2}\, c_\mathrm{A}\, c_\mathrm{C}, \tag{2.47}$$

$$\frac{\mathrm{d}c_\mathrm{AB}}{\mathrm{d}t} = -k_1\, c_\mathrm{AB} + k_{-1}\, c_\mathrm{A}\, c_\mathrm{B}, \tag{2.48}$$

$$\frac{\mathrm{d}c_\mathrm{AC}}{\mathrm{d}t} = -k_2\, c_\mathrm{AC} + k_{-2}\, c_\mathrm{A}\, c_\mathrm{C}. \tag{2.49}$$

You can verify conservation of total A, B, and C, respectively by showing that

$$\frac{\mathrm{d}c_\mathrm{A}}{\mathrm{d}t} + \frac{\mathrm{d}c_\mathrm{AB}}{\mathrm{d}t} + \frac{\mathrm{d}c_\mathrm{AC}}{\mathrm{d}t} = 0, \tag{2.50}$$

$$\frac{\mathrm{d}c_\mathrm{B}}{\mathrm{d}t} + \frac{\mathrm{d}c_\mathrm{AB}}{\mathrm{d}t} = 0, \tag{2.51}$$

$$\frac{\mathrm{d}c_\mathrm{C}}{\mathrm{d}t} + \frac{\mathrm{d}c_\mathrm{AC}}{\mathrm{d}t} = 0. \tag{2.52}$$

2.8 Rate laws should be empirically determined

Mass action gives good expressions for reaction rates *when all of the reactions and intermediate reactions are known*. We may not always know the complete set of **elementary steps** that make up a reactions scheme, and then we can naively apply mass action kinetics to the wrong set of reactions. It is therefore important to measure chemical kinetics experimentally to obtain phenomenological rate laws from which we can infer commensurate mechanisms.

Let's see how this can lead to trouble with a simple example that will also serve as motivation to some of the techniques we will present in the next lesson for solving mass action dynamical equations. Say we have a protein that we know has two configurations, A and B. We start with all proteins in configuration A, and then suddenly change buffer conditions so that it may switch to configuration B. We might write the chemical reaction as

$$\mathrm{A} \xrightarrow{k} \mathrm{B}. \tag{2.53}$$

This would give an expression for the dynamics of the concentration of B of

$$\frac{\mathrm{d}c_\mathrm{B}}{\mathrm{d}t} = k\, c_\mathrm{A}. \tag{2.54}$$

Empirically, you instead find a rate law

$$\frac{\mathrm{d}c_\mathrm{B}}{\mathrm{d}t} = k\, c_\mathrm{A}^2. \tag{2.55}$$

How could this be? Most likely, when the empirically derived rate law does not match what you wrote down using mass action kinetics, there could be intermediate reactions you are not considering. For example, it could be that A must first dimerize before it can undergo its conformational change. So, the whole reaction scheme could be

$$\mathrm{A} + \mathrm{A} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{AA}, \tag{2.56}$$

$$\mathrm{AA} \xrightarrow{k_2} \mathrm{A} + \mathrm{B}. \tag{2.57}$$

So, our mass action rate expressions are

$$\frac{\mathrm{d}c_\mathrm{A}}{\mathrm{d}t} = -2k_1\, c_\mathrm{A}^2 + 2k_{-1}\, c_\mathrm{AA} + k_2\, c_\mathrm{AA}, \tag{2.58}$$

$$\frac{\mathrm{d}c_\mathrm{AA}}{\mathrm{d}t} = k_1\, c_\mathrm{A}^2 - k_{-1}\, c_\mathrm{AA} - k_2\, c_\mathrm{AA}, \tag{2.59}$$

$$\frac{\mathrm{d}c_\mathrm{B}}{\mathrm{d}t} = k_2\, c_\mathrm{AA}. \tag{2.60}$$

In order to solve for the dynamics of c_B, we will make the **quasi-steady state approximation** (QSSA), in which we assume that the intermediate species, AA, has a time derivative that is small compared to those of the other species. (We will talk more about the QSSA in the next lesson.) That is,

$$\frac{\mathrm{d}c_\mathrm{AA}}{\mathrm{d}t} \approx 0. \tag{2.61}$$

With this expression, we can solve for c_AA in terms of c_A.

$$\frac{\mathrm{d}c_\mathrm{AA}}{\mathrm{d}t} = k_1\, c_\mathrm{A}^2 - k_{-1}\, c_\mathrm{AA} - k_2\, c_\mathrm{AA} \approx 0 \;\Rightarrow\; c_\mathrm{AA} \approx \frac{k_1}{k_{-1} + k_2}\, c_\mathrm{A}^2. \tag{2.62}$$

Substituting this expression into the expression for $\mathrm{d}c_\mathrm{B}/\mathrm{d}t$ gives

$$\frac{\mathrm{d}c_\mathrm{B}}{\mathrm{d}t} = \frac{k_1 k_2}{k_{-1} + k_2}\, c_\mathrm{A}^2. \tag{2.63}$$

This is the empirically observed rate law, with $k = k_1 k_2/(k_{-1} + k_2)$, meaning that the proposed mechanism is commensurate with experiment. It is not necessarily *the* mechanism, but is *a* plausible mechanism that gives the observed rate behavior.

3 Analytical solution to kinetics equations

We have learned how to write rate equations in differential form. Ultimately, we usually measure concentrations, not time derivatives of concentrations. So, we would like to solve the differential equations that govern the dynamics of chemical systems. In the following examples, I show some techniques for achieving analytical solutions of the dynamical equations, including

- Solving first order linear differential equations
- Solving separable differential equations
- Using conservation of mass to reduce the number of differential equations
- Introducing extent of reaction and fractional conversion
- Making a quasi-steady state approximation

3.1 Simple decay

We already solved the example of simple decay in section 2.1. The dynamical equation is

$$\frac{\mathrm{d}c}{\mathrm{d}t} = -kc, \tag{3.1}$$

and the solution is

$$c(t) = c_0 \mathrm{e}^{-kt}. \tag{3.2}$$

We found the solution first, and then differentiated to get the differential equation. This differential equation is linear first order (and also separable), so it is easily solved. A plot of the solution for RNA degradation is sketched in Fig. 1.

3.2 Irreversible dimerization/cooperative degradation

In sections 2.2 and 2.3, we investigated irreversible dimerization. The chemical reaction dynamical equation is

$$\frac{\mathrm{d}c}{\mathrm{d}t} = -kc^2, \tag{3.3}$$

which is the same dynamical equation we would get for irreversible cooperative decay, $\mathrm{A} + \mathrm{A} \longrightarrow \emptyset$, in which two molecules much first bind each other to annihilate

themselves. (Here, c is the concentration of species A, and we have absorbed a factor of two into the traditional rate constant.) This is a separable differential equation that we can solve in a straightforward manner. Separating the equation, we have

$$\frac{\mathrm{d}c}{c^2} = -\mathrm{d}t\, k. \tag{3.4}$$

Integrating both sides yields

$$\int_{c_0}^{c} \mathrm{d}c' \frac{\mathrm{d}c'}{(c')^2} = -\int_0^t \mathrm{d}t'\, k \tag{3.5}$$

$$= -\frac{1}{c'}\bigg|_{c_0}^{c} = \frac{1}{c_0} - \frac{1}{c} = -\,kt'|_0^t = -kt. \tag{3.6}$$

Finally, rearranging gives

$$c(t) = \frac{c_0}{1 + c_0\, k\, t}. \tag{3.7}$$

We could plot this with software, but I think is is useful to sketch a solution. This helps us gain intuition and to make comparison to other rate laws. In particular, we would like to compare cooperative degradation with the simple degradation case we observed earlier. Recall that for simple degradation,

$$c(t) = c_0\, \mathrm{e}^{-kt}. \tag{3.8}$$

To make an apples-to-apples comparison, we can consider the case where they both have the same initial degradation rate. *Initially* we would observe linear decay for each mechanism, since for small t, we can expand the expressions as Taylor series.

$$\text{simple: } c(t) = c_0(1 - kt + \ldots) \tag{3.9}$$

$$\text{cooperative: } c(t) = c_0(1 - c_0 kt + \ldots). \tag{3.10}$$

So, we want to compare k for simple degradation to $c_0 k$ for cooperative degradation. So, in our plot containing each of the two curves, we should plot c/c_0 versus kt or $c_0 kt$. Using the rule of thumb for plotting exponential decay, the concentration decays by a factor of about one-third for every time unit $1/k$. Conversely, it takes two $1/c_0 k$ time units to decay to one-third of the initial concentration for the cooperative mechanism. This can be seen by calculating

$$\frac{c(t)}{c_0} = \frac{1}{3} = \frac{1}{1 + c_0 kt} \;\Rightarrow\; t = \frac{2}{c_0 k}. \tag{3.11}$$

It takes two $1/k$ time units to decay to about one-ninth exponentially, but *eight* $1/c_0 k$ time units for cooperative degradation. So, cooperative degradation happens much more slowly! This is shown in the sketch in Fig. 2. This is an example of key insight afforded by analytically solving for the kinetics.

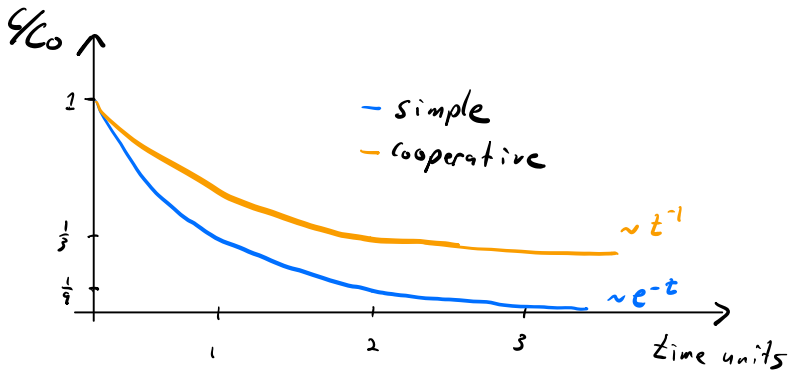


Figure 2: Concentration as a fraction of initial concentration due to simple decay (blue) and cooperative degradation (orange). The time axis is in units of k^{-1} for simple decay and in units of $(c_0 k)^{-1}$ for cooperative degradation.

3.3 Reversible dimerization

Now we will consider the case where the dimerization reaction is reversible, such that the chemical reaction is $\mathrm{A} + \mathrm{A} \underset{k_-}{\overset{k_+}{\rightleftharpoons}} \mathrm{AA}$. This analytical solution is more conplicated and difficult to obtain. This section is mostly included to help motivate numerical solutions for chemical dynamics, so you can skip this section if you like.

The dynamics for reversible dimerization are

$$\frac{\mathrm{d}c_{\mathrm{AA}}}{\mathrm{d}t} = k_+ c_{\mathrm{A}}^2 - k_- c_{\mathrm{AA}}, \tag{3.12}$$

$$\frac{\mathrm{d}c_{\mathrm{A}}}{\mathrm{d}t} = -2k_+ c_{\mathrm{A}}^2 + 2k_- c_{\mathrm{AA}}. \tag{3.13}$$

We now have two coupled differential equations to solve. However, we know mass must be conserved. If c_{A}^0 is the total concentration of A units, then

$$c_{\mathrm{A}}^0 = c_{\mathrm{A}} + 2c_{\mathrm{AA}}. \tag{3.14}$$

We can insert $c_{\mathrm{AA}} = (c_{\mathrm{A}}^0 - c_{\mathrm{A}})/2$ into the expression for $\mathrm{d}c_{\mathrm{A}}/\mathrm{d}t$ to get

$$\frac{\mathrm{d}c_{\mathrm{A}}}{\mathrm{d}t} = -2k_+ c_{\mathrm{A}}^2 + k_-(c_{\mathrm{A}}^0 - c_{\mathrm{A}}) = -2k_+ c_{\mathrm{A}}^2 - k_- c_{\mathrm{A}} + k_- c_{\mathrm{A}}^0. \tag{3.15}$$

This is again separable,

$$\int_{c_{\mathrm{A},0}}^{c_{\mathrm{A}}} \frac{\mathrm{d}c'_{\mathrm{A}}}{-2k_+ c_{\mathrm{A}}^2 - k_- c_{\mathrm{A}} + k_- c_{\mathrm{A}}^0} = \int_0^t \mathrm{d}t' = t, \tag{3.16}$$

where $c_{\mathrm{A},0}$ is the initial concentration of monomeric A (not the *total* concentration of A; that is c_{A}^0). To perform the integral, we use an integral table to look up the result

of

$$\int \frac{\mathrm{d}x}{ax^2+bx+c}$$

for the case where $4ac - b^2 < 0$, which is what we have here, since $a = -2k_+$, $b = -k_-$, and $c = k_- \, c_\mathrm{A}^0$, giving $4ac - b^2 = -8k_+k_- \, c_A^0 - k_-^2 < 0$. The result is

$$\int \frac{\mathrm{d}x}{ax^2+bx+c} = \begin{cases} -\frac{2}{\sqrt{b^2-4ac}} \operatorname{arctanh} \frac{2ax+b}{\sqrt{b^2-4ac}} & \text{for } \frac{|2ax+b|}{\sqrt{b^2-4ac}} < 1 \\ -\frac{2}{\sqrt{b^2-4ac}} \operatorname{arccoth} \frac{2ax+b}{\sqrt{b^2-4ac}} & \text{for } \frac{|2ax+b|}{\sqrt{b^2-4ac}} \geq 1. \end{cases} \tag{3.17}$$

For convenience, we define

$$\zeta = \left(1 + 8\,\frac{k_+}{k_-}\,c_\mathrm{A}^0\right)^{-\frac{1}{2}}, \tag{3.18}$$

such that, in the above expression,

$$\sqrt{b^2-4ac} = \frac{k_-}{\zeta}. \tag{3.19}$$

We also note that in the above expression,

$$2ax + b = -4k_+c_\mathrm{A} - k_- = -k_-\left(1 + 4\,\frac{k_+}{k_-}\,c_\mathrm{A}\right), \tag{3.20}$$

such that

$$\frac{2ax+b}{\sqrt{b^2-4ac}} = -\zeta\left(1 + 4\,\frac{k_+}{k_-}\,c_\mathrm{A}\right). \tag{3.21}$$

We define $f(c_\mathrm{A})$ to be

$$f(c_\mathrm{A}) = \begin{cases} \operatorname{arctanh}\left[\zeta\left(1 + 4\,\frac{k_+}{k_-}\,c_\mathrm{A}\right)\right] & \text{for } \zeta\left(1 + 4\,\frac{k_+}{k_-}\,c_\mathrm{A}\right) < 1. \\ \operatorname{arccoth}\left[\zeta\left(1 + 4\,\frac{k_+}{k_-}\,c_\mathrm{A}\right)\right] & \text{for } \zeta\left(1 + 4\,\frac{k_+}{k_-}\,c_\mathrm{A}\right) \geq 1. \end{cases} \tag{3.22}$$

Then, we have

$$f(c_\mathrm{A}) - f(c_{\mathrm{A},0}) = \frac{k_-t}{2\zeta}. \tag{3.23}$$

This gives an implicit equation for c_A as a function of t. The problem with this expression is that for a given t, c_A is multivalued. The value of c_A cannot jump from

the hyperbolic arctangent part of the solution to the hyperbolic arccotangent part of the solution. So, the initial condition dictates whether hyperbolic arctangents or arccotangents are involved in the solution. Therefore, the solution is

$$c_{\mathrm{A}}(t) = \frac{1}{4}\frac{k_-}{k_+}\left(\zeta^{-1}\tanh\left[\frac{k_- t}{2\zeta} + \operatorname{arctanh}\left(\zeta\left(1 + 4\,\frac{k_+}{k_-}\,c_{\mathrm{A},0}\right)\right)\right] - 1\right) \tag{3.24}$$

if

$$\zeta\left(1 + 4\,\frac{k_+}{k_-}\,c_{\mathrm{A},0}\right) < 1 \tag{3.25}$$

and

$$c_{\mathrm{A}}(t) = \frac{1}{4}\frac{k_-}{k_+}\left(\zeta^{-1}\coth\left[\frac{k_- t}{2\zeta} + \operatorname{arccoth}\left(\zeta\left(1 + 4\,\frac{k_+}{k_-}\,c_{\mathrm{A},0}\right)\right)\right] - 1\right) \tag{3.26}$$

if

$$\zeta\left(1 + 4\,\frac{k_+}{k_-}\,c_{\mathrm{A},0}\right) \geq 1. \tag{3.27}$$

I show this solution not because it is illuminating; it isn't really. I show it because even for simple-looking reactions, the nonlinearities that present themselves in mass action rate expressions can lead to difficult to find and difficult to interpret solutions. This motivates numerical solution of the dynamical equations, which is the topic of the next lesson.

I will note, however, that with some simplifying assumptions and in some limits, we can make approximate analytical progress, and that is helpful. We will not do that here, but may see some examples later in class where this is useful.

3.4 Irreversible binding of two different species

Consider now a new reaction, the irreversible binding of two species, $\mathrm{A}+\mathrm{B} \xrightarrow{k} \mathrm{AB}$. The dynamical equations are

$$\frac{\mathrm{d}c_{\mathrm{A}}}{\mathrm{d}t} = \frac{\mathrm{d}c_{\mathrm{B}}}{\mathrm{d}t} = -kc_{\mathrm{A}}c_{\mathrm{B}}. \tag{3.28}$$

These can be cast into a single equation as follows. We define a new variable Φ, which we will call the **extent of reaction**. Generally, for species j in a chemical reaction i, the extent of reaction i is defined as

$$\Phi_i(t) = \frac{N_j(t) - N_{j,0}}{\nu_{ij}}, \tag{3.29}$$

where N_j is the number of particles of species j and $N_{j,0}$ is the number of particles of species j initially present. Note that $\Phi_i(t)$ has the same value for all species j. We define $\phi_i = \Phi_i/V$, where V is the volume of the solution, assumed to be constant, giving

$$\phi_i(t) = \frac{c_j(t) - c_{j,0}}{\nu_{ij}}. \tag{3.30}$$

Then, for the binding reaction we are considering at present, we can write

$$c_\mathrm{A} = c_{\mathrm{A},0} - \phi, \tag{3.31}$$

$$c_\mathrm{B} = c_{\mathrm{B},0} - \phi, \tag{3.32}$$

since the stoichiometric coefficient for both A and B is -1. Now, we get a single differential equation (since the time derivative of constants like $c_{\mathrm{A},0}$ are zero),

$$\frac{\mathrm{d}\phi}{\mathrm{d}t} = -k(c_{\mathrm{A},0} - \phi)(c_{\mathrm{B},0} - \phi). \tag{3.33}$$

This is separable, and we can write it as

$$\frac{\mathrm{d}\phi}{(c_{\mathrm{A},0} - \phi)(c_{\mathrm{B},0} - \phi)} = -\mathrm{d}t\, k. \tag{3.34}$$

To integrate the left hand side, we can do a partial fraction expansion of the integrand.

$$\begin{aligned}\int_0^\phi \frac{\mathrm{d}\phi'}{(c_{\mathrm{A},0} - \phi')(c_{\mathrm{B},0} - \phi')} &= \int_0^\phi \mathrm{d}\phi' \frac{1}{c_{\mathrm{B},0} - c_{\mathrm{A},0}}\left(\frac{1}{c_{\mathrm{A},0} - \phi'} - \frac{1}{c_{\mathrm{B},0} - \phi'}\right) \\ &= \frac{1}{c_{\mathrm{B},0} - c_{\mathrm{A},0}} \ln\left(\frac{c_{\mathrm{A},0} - \phi'}{c_{\mathrm{B},0} - \phi'}\right)\Bigg|_0^\phi \\ &= \frac{1}{c_{\mathrm{B},0} - c_{\mathrm{A},0}}\left[\ln\left(\frac{c_{\mathrm{A},0} - \phi}{c_{\mathrm{B},0} - \phi}\right) - \ln\left(\frac{c_{\mathrm{A},0}}{c_{\mathrm{B},0}}\right)\right].\end{aligned} \tag{3.35}$$

Integrating the right hand side yields

$$-\int_0^t \mathrm{d}t'\, k = -kt, \tag{3.36}$$

such that

$$\frac{1}{c_{\mathrm{B},0} - c_{\mathrm{A},0}}\left[\ln\left(\frac{c_{\mathrm{A},0} - \phi}{c_{\mathrm{B},0} - \phi}\right) - \ln\left(\frac{c_{\mathrm{A},0}}{c_{\mathrm{B},0}}\right)\right] = -kt. \tag{3.37}$$

Rearranging, we have

$$\ln\left(\frac{c_{\mathrm{A},0}-\phi}{c_{\mathrm{B},0}-\phi}\right)=\ln\left(\frac{c_{\mathrm{A},0}}{c_{\mathrm{B},0}}\right)-k(c_{\mathrm{B},0}-c_{\mathrm{A},0})t. \tag{3.38}$$

Evidently, the logarithm of the ratio of the concentrations of A and B decays linearly with time. This can be solved for ϕ to give

$$\phi(t)=\frac{c_{\mathrm{A},0}-w\,c_{\mathrm{B},0}}{1-w}, \tag{3.39}$$

where

$$w(t)=\frac{c_{\mathrm{A},0}}{c_{\mathrm{B},0}}\,\mathrm{e}^{-k(c_{\mathrm{B},0}-c_{\mathrm{A},0})t}. \tag{3.40}$$

The concentrations $c_{\mathrm{A}}(t)$ and $c_{\mathrm{B}}(t)$ may then be calculated using

$$c_{\mathrm{A}}(t)=c_{\mathrm{A},0}-\phi(t), \tag{3.41}$$

$$c_{\mathrm{B}}(t)=c_{\mathrm{B},0}-\phi(t). \tag{3.42}$$

Note that this solution only works when $c_{\mathrm{A},0}\neq c_{\mathrm{B},0}$. When $c_{\mathrm{A},0}=c_{\mathrm{B},0}$, the situation is identical mathematically to the dimerization case treated above in section 3.2 with the result that

$$c_{\mathrm{A}}(t)=c_{\mathrm{B}}(t)=\frac{c_0}{1+c_0kt}, \tag{3.43}$$

where $c_0=c_{\mathrm{A},0}=c_{\mathrm{B},0}$.

This approach does lead to a messy expression, but is still of some utility beyond a numerical solution because it gives the insight that the logarithm of the ratio of the concentrations of A and B decays linearly with time.

3.4.1 Fractional conversion

The extent of reaction Φ is a useful quantity, but suffers from the fact that is is **extensive**, meaning that it is proportional to the total number of molecules present in your reaction volume. We could instead define **fractional conversion** due to reaction i, ξ_i, to be the ratio of the extent of reaction to the maximum possible extent of reaction,

$$\xi_i(t)=\frac{\Phi_i(t)}{\Phi_{i,\max}}, \tag{3.44}$$

where

$$\Phi_{i,\max} = -\frac{N_l^0}{\nu_{il}}, \tag{3.45}$$

where the subscript l here denotes the limiting reagent for reaction i. Then,

$$\xi_i(t) = \frac{\Phi_i(t)}{\Phi_{i,\max}} = -\nu_{il}\,\frac{N_j(t) - N_j^0}{\nu_{ij}\,N_l^0}. \tag{3.46}$$

In the case where the reaction is carried out at constant volume V, we can divide both the numerator and denominator by V to get

$$\xi_i(t) = -\nu_{il}\,\frac{c_j(t) - c_j^0}{\nu_{ij}\,c_l^0}. \tag{3.47}$$

This quantity is proportional to the extent of reaction, but conveniently is dimensionless, ranges from zero to one, and has the meaning of the fraction of the total possible progress of a chemical reaction. We could have used fractional conversion in the above analysis, and will employ it in future applications we will encounter.

3.5 The quasi-steady state approximation

We have seen the quasi-steady state approximation (QSSA) put to use in section 2.8. This approximation is one of the most useful, accurate, and widely-used (and maybe also abused) approximations to tame systems of ordinary differential equations arising from mass action kinetics to analytical tractability. We will put it to use again when we study enzyme kinetics.

To understand the idea behind the QSSA, we first define a **reactive intermediate** as a chemical species that is not one of the stable products or reactants of a reaction, but rather is present in small concentrations with short lifetimes. *The QSSA involves setting the time derivative of reactive intermediates to zero.* Because reactive intermediates cannot accumulate in large amounts, their time derivatives cannot grow as large as more stable species.

The equation that results from setting the time derivative of a reactive intermediate to zero is an *algebraic* equation. Usually, this gives an expression for the concentration of a reactive intermediate in terms of a more stable reactant, thereby enabling an expression of the time derivative of product concentrations only in terms of the concentrations of more stable reactants.

With this description of the QSSA in mind, re-read section 2.8 to see an example of how it is applied.

In addition to reactive intermediates, the QSSA is also applicable to chemical species that are in very large excess.

4 Numerical solution to kinetics equations

In this lesson we discuss numerical methods for solving chemical kinetics equations. This is best done in a Jupyter notebook, which may be accessed here.

5 Enzyme kinetics

We have seen how to translate from a set of chemical reactions to a system of differential equations describing the dynamics of the concentrations of the chemical species. We have also seen how solve these ODEs analytically for a subset of those that we can, and we have also learned how to solve these ODEs numerically. These techniques are general; given a set of chemical reactions, we can write a system of ODEs using the principle of mass action, and can then solve them (stiffness and other numerical issues aside).

In this lesson, we will discuss **enzymes** (defined below), and we can and will apply those techniques to systems of ODEs arising from enzyme kinetics. We will also show that making appropriate approximations to derive analytical expressions for enzyme dynamics will be useful for conceptual understanding. This cannot be overstated: While numerical solutions of ODEs (or other equations)are very useful, significant insight can be gained by making (sometimes approximate) analytical progress.

5.1 Catalysts and enzymes

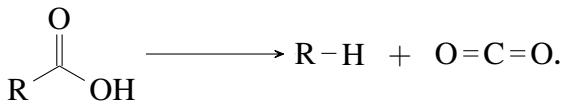
A **catalyst** is a chemical substance that increases the rate of a chemical reaction without itself being consumed in the process. In industrial applications, catalysts have many immeasurable impacts. Perhaps the most famous is the Haber-Bosch process, in which a metal (usually iron) catalyst allows for rapid, inexpensive production of ammonia, which is widely used in production of fertilizer. Haber-Bosch chemistry is responsible for about 2% of the world's energy usage and 5% of natural gas production is used in this process. In many ways, it is this catalyzed chemistry that has led to the population explosion over the last 100 years.

While humans have been successful in engineering (mostly metal-based) catalysts, the living world has evolved an amazing variety of effective catalysts. Most of the catalysts active in living systems are protein-based. Such catalysts are called **enzymes**. RNAs, and RNA-protein complexes can also serve as catalysts, and other biomolecules may as well. Enzymes work on other molecules, termed **substrates**. These are the chemical species involved in the reactions that the enzymes catalyze.

Enzyme catalysts can have an extraordinary effect on reaction rates.[6] As a typical example, consider peptide hydrolysis, the degradation of peptide bonds the covalently connect amino acids in peptide chains. At 25°C this reaction is quite slow, with a half time (the time it takes for half of a set of peptide bonds to be hydrolyzed) of about 450 years. By contrast, this reaction proceeds much faster with the help

[6] For a nice review, check out Wolfenden and Snider, *Acc. Chem. Red.*, **34**, 938–945.

of a carboxypeptidase enzyme, with a half time of about 2 milliseconds. That is a difference of about 13 orders of magnitude! As an extreme example, consider the decarboxylation of an amino acid,



This reaction has a half time of about a billion years at 25°C in water. The arginine decarboxylase enzyme brings this half time down to about a millisecond, about a 10^{19} fold speed boost!

The mechanisms by which there speed boosts are obtained (mainly by lowering activation energies) are fascinating subjects, but outside of the scope of this course's very short treatment of chemical reaction kinetics. For now, we will investigate chemical reaction schemes by which enzymes work and derive expressions for the dynamics of substrate depletion and product formation for enzyme catalyzed reactions.

5.2 Michaelis-Menten kinetics

In a seminal paper in 1913, Leonor Michaelis and Maud Menten studied the action of invertase, an enzyme that converts sucrose to fructose and glucose. In their paper, they proposed a simple mechanism for enzyme catalyzed reactions in which the enzyme reversibly binds to the substrate The enzyme-substrate complex can then irreversibly be converted to the product, freeing the enzyme. The scheme may be written as

$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES} \xrightarrow{k_2} \mathrm{E} + \mathrm{P}. \tag{5.1}$$

From this reaction scheme, we may write down the corresponding system of ODEs according to mass action.

$$\frac{\mathrm{d}c_\mathrm{S}}{\mathrm{d}t} = -k_1\, c_\mathrm{E}\, c_\mathrm{S} + k_{-1}\, c_\mathrm{ES}, \tag{5.2}$$

$$\frac{\mathrm{d}c_\mathrm{ES}}{\mathrm{d}t} = k_1\, c_\mathrm{E}\, c_\mathrm{S} - (k_{-1} + k_2) c_\mathrm{ES}, \tag{5.3}$$

$$\frac{\mathrm{d}c_\mathrm{P}}{\mathrm{d}t} = k_2\, c_\mathrm{ES}, \tag{5.4}$$

$$\frac{\mathrm{d}c_\mathrm{E}}{\mathrm{d}t} = -k_1\, c_\mathrm{E}\, c_\mathrm{S} + (k_{-1} + k_2) c_\mathrm{ES}. \tag{5.5}$$

We can leave these equations as they are and solve them numerically for some initial condition if we wish. Alternatively, we can make some simplifications and write perhaps clearer expressions.

Michaelis and Menten did not make the QSSA; this was done later in 1925 by Briggs and Haldane. Rather, they assumed that the enzyme-substrate binding reaction achieved a rapid equilibrium. We will instead proceed with a QSSA, setting $\mathrm{d}c_{\mathrm{ES}}/\mathrm{d}t = 0$.

$$\frac{\mathrm{d}c_{\mathrm{ES}}}{\mathrm{d}t} = k_1\, c_{\mathrm{E}}\, c_{\mathrm{S}} - (k_{-1} + k_2) c_{\mathrm{ES}} = 0. \tag{5.6}$$

By conservation of enzyme, we have that

$$c_{\mathrm{E}}^0 = c_{\mathrm{E}} + c_{\mathrm{ES}}, \tag{5.7}$$

where c_{E}^0 is the total concentration of free and bound enzyme. Thus, $c_{\mathrm{E}} = c_{\mathrm{E}}^0 - c_{\mathrm{ES}}$. Substituting this relation into the QSSA expression above and solving for c_{ES} yields

$$c_{\mathrm{ES}} = \frac{k_1\, c_{\mathrm{E}}^0\, c_{\mathrm{S}}}{k_1\, c_{\mathrm{S}} + k_{-1} + k_2} = c_{\mathrm{E}}^0\, \frac{c_{\mathrm{S}}/K_{\mathrm{M}}}{1 + c_{\mathrm{S}}/K_{\mathrm{M}}}, \tag{5.8}$$

where

$$K_{\mathrm{M}} \equiv \frac{k_{-1} + k_2}{k_1} \tag{5.9}$$

is called the **Michaelis constant**.[7] Substituting this into the expression for $\mathrm{d}c_{\mathrm{P}}/\mathrm{d}t$ gives

$$\frac{\mathrm{d}c_{\mathrm{P}}}{\mathrm{d}t} = k_2 c_{\mathrm{E}}^0\, \frac{c_{\mathrm{S}}/K_{\mathrm{M}}}{1 + c_{\mathrm{S}}/K_{\mathrm{M}}}. \tag{5.10}$$

This equation is known as the **Michaelis-Menten equation**. It is often written in terms of **reaction velocity**, v_0, which is simply $\mathrm{d}c_{\mathrm{P}}/\mathrm{d}t$, as

$$\frac{\mathrm{d}c_{\mathrm{P}}}{\mathrm{d}t} \equiv v_0 = v_{\max}\, \frac{c_{\mathrm{S}}/K_{\mathrm{M}}}{1 + c_{\mathrm{S}}/K_{\mathrm{M}}}, \tag{5.11}$$

where $v_{\max} = k_2\, c_{\mathrm{E}}^0$.

5.2.1 Properties of the Michaelis-Menten equation

We now investigate the properties of the Michelis-Menten equation. A sketch of reaction rate (referred to as "velocity" in the context of enzyme catalyzed reactions)

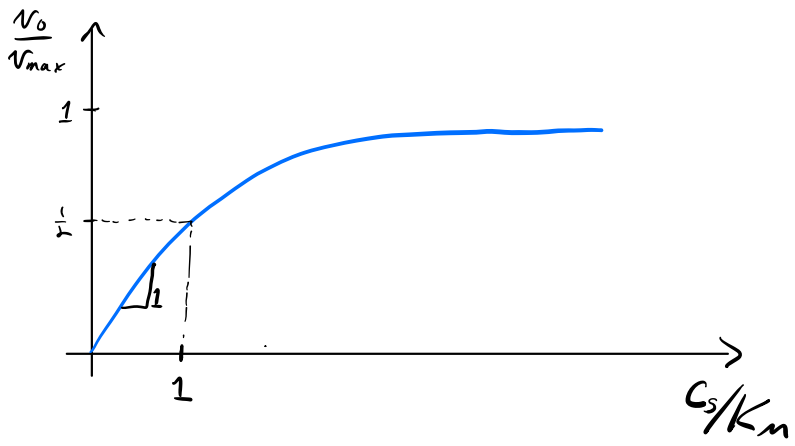


Figure 3: The velocity $v_0 = \mathrm{d}c_P/\mathrm{d}t$ versus substrate concentration in units of the Michaelis constant for Michaelis-Menten kinetics.

versus substrate concentration is shown in Fig. 3. In looking at the plot, recall that $v_{max} = k_2\, c_E^0$, proportional to the total enzyme concentration.

In studying the plot and the Michaelis-Menten equation, a few key properties are apparent.

1. The maximum velocity is proportional to the total enzyme concentration. The reaction goes faster as more enzyme is available.
2. The Michaelis constant K_M sets the scale of the substrate concentration necessary to achieve a high reaction velocity. If the substrate concentration is far greater than the Michaelis constant, the velocity is approximately v_{max}. In this high substrate concentration regime, the enzyme is said to be **operating at saturation**. The kinetics are no longer dependent on substrate concentration and are instead limited by how much enzyme is available to serve as a catalyst.
3. In general, the **catalytic rate constant**, k_{cat} is defined such that $v_{max} = k_{cat}\, c_E^0$. The catalytic rate constant has dimension of inverse time and is also referred to as the **turnover number**, as it is proportional to the number of reactions per unit time a single enzyme molecule (or mole of enzyme, micromole of enzyme, etc., depending on the chosen units of c_E^0) can perform. For the Michaelis-Menten mechanism, $k_{cat} = k_2$.
4. For substrate concentrations much smaller than the Michaelis constant, the velocity grows linearly with substrate concentration with slope $k_2\, c_E^0/K_M$. Thus, at low substrate concentration, k_2/K_M (or more generally k_{cat}/K_M) serves as

[7]There are many ways to define a Michaelis constant, depending on reaction scheme and how a QSSA or other approximation is applied. This is one of them.

an effective rate constant, since

$$\frac{\mathrm{d}c_\mathrm{P}}{\mathrm{d}t} \equiv v_0 \approx \frac{k_\mathrm{cat}}{K_\mathrm{M}}\, c_\mathrm{E}^0\, c_\mathrm{S} \tag{5.12}$$

in the low substrate concentration limit. The quantity $k_\mathrm{cat}/K_\mathrm{M}$ is referred to as the **catalytic efficiency** of the enzyme. The bigger the catalytic efficiency, the faster the enzyme can perform the reaction.

5. You may notice that the substrate concentration-dependence is in the ubiquitous form of the Langmuir isotherm and **Hill function**, $(c_\mathrm{S}/K_\mathrm{M})/(1+c_\mathrm{S}/K_\mathrm{M})$.

5.2.2 Solving the Michaelis-Menten equation

The Michaelis-Menten equation is a convenient expression for the *rate* of production of product, but we can make further progress, and eventually solve the resulting ODE, if we instead look at the rate of consumption of substrate. We can use the conservation law of total substrate product,

$$\frac{\mathrm{d}}{\mathrm{d}t}\left(c_\mathrm{S} + c_\mathrm{ES} + c_\mathrm{P}\right) = 0. \tag{5.13}$$

Since we used the QSSA such that $\mathrm{d}c_\mathrm{ES}/\mathrm{d}t = 0$, we have that

$$\frac{\mathrm{d}c_\mathrm{S}}{\mathrm{d}t} = -\frac{\mathrm{d}c_\mathrm{P}}{\mathrm{d}t} = -k_2 c_\mathrm{E}^0\, \frac{c_\mathrm{S}/K_\mathrm{M}}{1 + c_\mathrm{S}/K_\mathrm{M}}. \tag{5.14}$$

This differential equation is again separable, as we have seen with many of the ODEs arising from mass action kinetics we have encountered.

$$\mathrm{d}c_\mathrm{S}\, \frac{1 + c_\mathrm{S}/K_\mathrm{M}}{c_\mathrm{S}/K_\mathrm{M}} = -\mathrm{d}t\, k_2 c_\mathrm{E}^0. \tag{5.15}$$

The right hand side may be easily integrated to give

$$-\int_0^t \mathrm{d}t'\, k_2 c_\mathrm{E}^0 = -k_2\, c_\mathrm{E}^0\, t. \tag{5.16}$$

We can integrate the left hand side as follows.

$$\begin{aligned}\int_{c_\mathrm{S}^0}^{c_\mathrm{S}} \mathrm{d}c'_\mathrm{S}\, \frac{1 + c'_\mathrm{S}/K_\mathrm{M}}{c'_\mathrm{S}/K_\mathrm{M}} &= \int_{c_\mathrm{S}^0}^{c_\mathrm{S}} \mathrm{d}c'_\mathrm{S} \left(1 + \frac{K_\mathrm{M}}{c'_\mathrm{S}}\right) \\ &= c_\mathrm{S} - c_\mathrm{S}^0 + K_\mathrm{M} \int_{c_\mathrm{S}^0}^{c_\mathrm{S}} \frac{\mathrm{d}c'_\mathrm{S}}{c'_\mathrm{S}}\end{aligned} \tag{5.17}$$

$$= c_S - c_S^0 + K_M \ln \frac{c_S}{c_S^0}$$

Thus, we have

$$c_S + K_M \ln \frac{c_S}{c_S^0} = c_S^0 - k_2 c_E^0 t. \tag{5.18}$$

This is easier to work with if we divide by the Michaelis constant (putting concentrations in units of the Michaelis constant).

$$\frac{c_S}{K_M} + \ln \frac{c_S}{c_S^0} = \frac{c_S^0}{K_M} - k_2 \frac{c_E^0}{K_M} t. \tag{5.19}$$

There is no closed-form solution for c_S as a function of time, but the above relation is useful. First, we see that the initial condition is satisfied. When $t = 0$, we have the $c_S(t = 0) = c_S^0$. For short times, we still have $c_S \approx c_S^0$, such that $\ln(c_S/c_S^0) \approx 0$, giving

$$c_S \approx c_S^0 - k_2 c_E^0 t. \tag{5.20}$$

This is what we would expect from the Michaelis-Menten differential equation (5.10); for short times, when substrate is abundant, substrate is depleted (and therefore product formed) at a rate proportional to $v_{max} = k_2 c_E^0$. For long times, as $t \to \infty$, the right hand side tends toward $-\infty$. The only way the left hand side can also tend toward $-\infty$ is if c_s tends toward zero. This tells us that eventually all of the substrate will be consumed, as we would expect due to the irreversible final reaction in the scheme.

We can continue and write a solution in terms of the Lambert W function. This is actually of less use than integrating the Michaelis-Menten system of ODEs numerically (since that is done without invoking a QSSA or other approximation), but I show it here for completeness. Exponentiating both sides gives

$$\frac{c_S}{c_S^0} e^{c_S/K_M} = e^{(c_S^0 - k_2 c_E^0 t)/K_M}. \tag{5.21}$$

Multiplying both sides by c_S^0/K_M gives

$$\frac{c_S}{K_M} e^{c_S/K_M} = \frac{c_S^0}{K_M} e^{(c_S^0 - k_2 c_E^0 t)/K_M}. \tag{5.22}$$

The primary branch of the Lambert W function, $W_0(x)$, is the solution y to $y\, e^y = x$, where x is positive and x and y are both real. Thus,

$$\frac{c_S}{K_M} = W_0\left(\frac{c_S^0}{K_M} e^{(c_S^0 - k_2 c_E^0 t)/K_M}\right). \tag{5.23}$$

If we define c_S^0 to be the total amount of substrate, bound to enzyme or unbound, and assume we initially have no product ($c_P(0) = 0$), then, by conservation of total substrate and product, we have

$$c_P = c_S^0 - c_S - c_{ES}. \tag{5.24}$$

We can use the above expression for $c_S(t)$ and our expression for c_{ES} obtained from the QSSA in equation (5.8), to get

$$c_P(t) = c_S^0 - c_S - c_E^0 \frac{c_S/K_M}{1 + c_S/K_M}, \tag{5.25}$$

where c_S is given by equation (5.23).

5.3 Inhibition

In industrial processes, catalysts can be **poisoned**, a process that usually involves adsorption of impurities in the feed. As a result, the catalysts have fewer available adsorption sites for reactant species, therefore slowing the reaction. Similarly, enzymes maybe "poisoned" when another molecule binds them rendering them incapably of binding substrate. In the enzyme setting, this is not called poisoning, but is called **inhibition**.

Here, we will consider the simple case of a single inhibitor that may bind an enzyme that is catalyzing a reaction that proceeds by the Michaelis-Menten mechanism. The set of chemical reactions now includes enzyme binding to inhibitor in addition to the Michaelis-Menten reactions.

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P, \tag{5.26}$$

$$E + I \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} EI. \tag{5.27}$$

To investigate the effect of the presence of an initiator on v_0, the rate at which product is produced, we use the usual procedure of writing differential equations according to mass action and apply the QSSA. Equations (5.2) through (5.4) still hold, and are reproduced below for clarity, but are supplemented by ODEs taking into account the inhibitor.

$$\frac{dc_S}{dt} = -k_1 c_E c_S + k_{-1} c_{ES}, \tag{5.28}$$

$$\frac{dc_{ES}}{dt} = k_1 c_E c_S - (k_{-1} + k_2) c_{ES}, \tag{5.29}$$

$$\frac{\mathrm{d}c_\mathrm{P}}{\mathrm{d}t} = k_2\, c_\mathrm{ES}, \tag{5.30}$$

$$\frac{\mathrm{d}c_\mathrm{E}}{\mathrm{d}t} = -(k_1\, c_\mathrm{S} + k_3\, c_\mathrm{I})c_\mathrm{E} + (k_{-1} + k_2)c_\mathrm{ES} + k_{-3}c_\mathrm{EI}, \tag{5.31}$$

$$\frac{\mathrm{d}c_\mathrm{I}}{\mathrm{d}t} = -k_3\, c_\mathrm{E}\, c_\mathrm{I} + k_{-3}c_\mathrm{EI}, \tag{5.32}$$

$$\frac{\mathrm{d}c_\mathrm{EI}}{\mathrm{d}t} = k_3\, c_\mathrm{E}\, c_\mathrm{I} - k_{-3}c_\mathrm{EI}. \tag{5.33}$$

Because $v_0 = k_2 c_\mathrm{ES}$, we seek an expression for c_ES in terms of c_E^0, c_S, and the total amount of inhibitor, c_I^0. We again can apply the QSSA to the dynamics of the ES intermediate such that $\mathrm{d}c_\mathrm{ES}/\mathrm{d}t \approx 0$, giving

$$c_\mathrm{ES} \approx \frac{k_1}{k_{-1} + k_2}\, c_\mathrm{E}\, c_\mathrm{S} = \frac{c_\mathrm{E}\, c_\mathrm{S}}{K_\mathrm{M}}, \tag{5.34}$$

exactly as in the uninhibited case. Without the inhibitor, we could use the conservation law for enzyme to write an expression for c_E in terms of the total enzyme concentration c_E^0 and the substrate concentration c_S, allowing use to write an expression for c_ES in terms of these two variables. This is exactly what we did in deriving the Michaelis-Menten equation. Now, however, the conservation law for enzyme is

$$c_\mathrm{E}^0 = c_\mathrm{E} + c_\mathrm{ES} + c_\mathrm{EI}, \tag{5.35}$$

such that

$$c_\mathrm{E} = c_\mathrm{E}^0 - c_\mathrm{ES} - c_\mathrm{EI}. \tag{5.36}$$

To make further analytical progress, we need an expression for c_EI. To do so, we need to make another approximation. We can assume that the binding and unbinding of inhibitor to enzyme reaches a **rapid equilibrium**, such that the net rate of the enzyme-inhibitor reaction is zero. That is, the forward and reverse rate of the enzyme-inhibitor binding reaction are equal, such that

$$k_3\, c_\mathrm{E}\, c_\mathrm{I} \approx k_{-3}c_\mathrm{EI}. \tag{5.37}$$

In general, a rapid equilibrium approximation assumes that the dynamics associated with a single reversible reaction are faster than the dynamics of interest, such that the forward and reverse rates of the reaction to which the approximation is applied are equal.

At this point, we can take two approaches. We can write c_EI in terms of the concentration of the inhibitor, c_I. This is what is commonly done, and is done in Kuriyan, Konforti, and Wemmer. Another approach is to write everything in terms

of c_{I}^0, which might be easier to control experimentally. The resulting expressions are messier in this case. In the first approach, we are treating the inhibitor similarly as the substrate; we write expressions in terms of c_{I} and c_{S} instead of c_{I}^0 and c_{S}^0. To keep things simple, we will use the first approach here.

From the rapid equilibrium approximation,

$$c_{\mathrm{EI}} \approx \frac{k_3}{k_{-3}}\, c_{\mathrm{E}}\, c_{\mathrm{I}}, \tag{5.38}$$

which gives

$$c_{\mathrm{E}} \approx c_{\mathrm{E}}^0 - c_{\mathrm{ES}} - \frac{k_3}{k_{-3}}\, c_{\mathrm{E}}\, c_{\mathrm{I}}. \tag{5.39}$$

We can solve this for the enzyme concentration c_{E},

$$c_{\mathrm{E}} = \frac{c_{\mathrm{E}}^0 - c_{\mathrm{ES}}}{1 + \frac{k_3}{k_{-3}}\, c_{\mathrm{I}}} \tag{5.40}$$

Substituting this expression into our expression for c_{ES} (equation 5.34), gives

$$c_{\mathrm{ES}} \approx \frac{c_{\mathrm{E}}^0 - c_{\mathrm{ES}}}{1 + \frac{k_3}{k_{-3}}\, c_{\mathrm{I}}}\, \frac{c_{\mathrm{S}}}{K_{\mathrm{M}}}. \tag{5.41}$$

Rearranging to solve for c_{ES}, this is

$$c_{\mathrm{ES}} \approx c_{\mathrm{E}}^0\, \frac{\frac{c_{\mathrm{S}}}{K_{\mathrm{M}}\left(1+\frac{k_3}{k_{-3}}\, c_{\mathrm{I}}\right)}}{1 + \frac{c_{\mathrm{S}}}{K_{\mathrm{M}}\left(1+\frac{k_3}{k_{-3}}\, c_{\mathrm{I}}\right)}}. \tag{5.42}$$

Apparently the inhibitor has introduced an effective Michaelis constant,

$$K_{\mathrm{M}}^* = K_{\mathrm{M}} \left(1 + \frac{k_3}{k_{-3}}\, c_{\mathrm{I}}\right). \tag{5.43}$$

which that we recover our usual Michaelis-Menten expression,

$$\frac{\mathrm{d}c_{\mathrm{P}}}{\mathrm{d}t} \equiv v_0 = k_2\, c_{\mathrm{ES}} \approx k_2\, c_{\mathrm{E}}^0\, \frac{c_{\mathrm{S}}/K_{\mathrm{M}}^*}{1 + c_{\mathrm{S}}/K_{\mathrm{M}}^*}. \tag{5.44}$$

If we have strong inhibition ($k_3 c_{\mathrm{I}}/k_{-3} \gg 1$), the effective Michaelis constant is much larger, thereby slowing the rate of the reaction.

6 Measurement of chemical rates

We have learned how to go from a putative reaction mechanism to differential equations describing the dynamics of concentrations of chemical species using mass action kinetics. Central to this analysis is the determination of the constants of proportionality between the product of concentrations of reactants and the reaction rate. We have come to know these as **rate constants**.

We are going to defer some of the theory behind rate constants, in particular concepts of temperature-dependence and diffusion-limited rates, until we have built the thermodynamic machinery we need to understand these concepts. For now, in this lesson, we will focus on methods for monitoring reactions such that kinetic rate constants may be *empirically* determined.

6.1 Monitoring reactions with light

Optical methods are among the most widely used for monitoring biochemical reactions. I will not go into detail about these methods here, but instead encourage you to read sections 2.6 and 2.7 of Wittrup, Tidor, Hackel, and Sarkar. Below is a brief summary of optical methods.

6.1.1 Scattering

When light impinges upon biomolecules, it can induce oscillations in the molecules. The oscillations result in a re-radiation, which is measured as scattered intensity. It is commonly used in flow cytometry to get a rough estimate of the size and shape of objects.

6.1.2 Absorbance

More than just scattering can happen when biomolecules are exposed to light. If the light has a wavelength (and therefore energy) corresponding to electronic transitions allowable in the molecule, the impinging photons may trigger the electronic transitions, and the photon is absorbed and does not exit the sample. By monitoring how much light gets through a sample, absorbance can be measured. For dilute solutions, the absorbance, given by $A = \ln(I_0/I)$, where I_0 is the intensity of incident light and I is the intensity of transmitted light, is directly proportional to absorbing species concentration, thereby allowing concentration determination.

6.1.3 Fluorescence

This is perhaps the most widely used method for monitoring quantity and location of biochemical species in use today. Some molecules can **fluoresce**. The process of fluorescence involves four steps.

1. **Excitation.** Incident light of wavelength corresponding to an excitation energy kicks the molecule into a higher energy excited electronic state. This happens on a femtosecond time scale.
2. **Excited radiationless transition.** While still in the excited electronic state, the molecule relaxes to a lower vibrational state. No photon is emitted in this transition. The lifetime of this state is on the order of nanoseconds, but can sometimes last as long as a microsecond.
3. **Emission.** The molecule relaxes back down to the ground electronic state. The difference in energy is accounted for by the release (emission) of a photon. Sometimes, a photon is not emitted and the energy is dissipated as heat instead. Emission happens on a femtosecond time scale.
4. **Ground radiationless transition.** As a final relaxation, the molecule drops to a low vibrational state within the ground electronic state. No photon is emitted.

Importantly, the time scale of the processes of fluorescence are much faster than most processes of biological interest. Unfortunately, not many molecules fluoresce, which is why those that do, called **fluorophores**, are often covalently bound to molecules of interest.

The photons emitted by fluorescence are measured by an assortment of detectors, including sophisticated microscopes that also allow for spatial data about the fluorophores.

Quenchers are molecules that, when in contact with an excited fluorophore, accept the energy from the excited state and rapidly convert it to heat, resulting in no photon emission. This process is called **quenching**. The efficiency E_{F} of quenching falls rapidly with the distance between the fluorophore and quencher according to

$$E_{\mathrm{F}} = \frac{1}{1 + (r - r_0)^6}, \tag{6.1}$$

where r is the distance between the fluorophore and quencher and r_0 is the **Förster radius**, named after Theodor Förster, who worked out the relation. As a result of this rapid decay of quenching efficiency with distance, molecules are often constructed with a fluorophore-quencher pair on the same molecule. This allows for experimental monitoring of conformations of biomolecules. Such experiments are called **FRET experiments**, an acronym for fluorescence (or Förster) resonance energy transfer.

6.2 Stopped flow experiments

There are many challenges for measuring the kinetic rate constants of fast reactions, such as those catalyzed by enzymes. One particularly daunting challenge is to completely mix the reactants without letting too much of the reaction proceed. For very slow reactions, one can simply pipette reactants into a cuvette and then measure absorbance, fluorescence, or whatever readout of species concentrations are applicable. But for fast reactions that proceed on time scales of milliseconds, this is very challenging.

In addition to relaxation experiments, described in the next section, **stopped flow experiments** provide a means to rapidly mix components and then quickly monitor optical properties of the reaction mixture over time. A schematic of a stopped flow apparatus is shown in Fig. 4. In this example, purified solutions of enzyme and substrate are added together via syringes into a microfluidic device. The device is designed to induce microscale turbulence immediately after the reactant streams come into contact, which accomplishes rapid mixing. After mixing, the reaction mixture enters a region called the sample cell, which has optically clear walls enabling spectroscopic detection. Typically the detectors can function at very high frequency, often in tens or hundreds of kHz. Once the input streams have mixed and entered the sample cell, the stop switch is triggered to stop the flow, and the reaction is monitored.

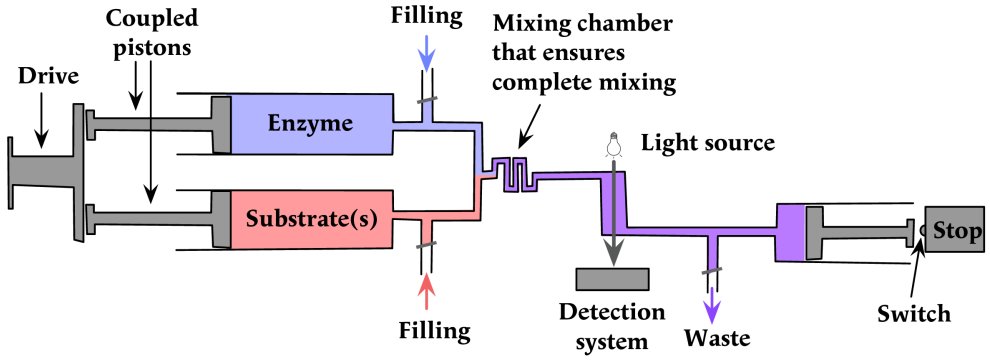


Figure 4: Set up of a stopped flow experiment for measuring enzyme kinetics. Image created by Athel Cornish-Bowden, licensed under a CC-BY-SA 4.0 license.

Ideally, stopped flow apparatuses have very low **dead time**, which is the amount of time that passes between the point when the reactant mixture first comes into contact and when it can be observed. Modern stopped flow devices can have dead times as low as hundreds of nanoseconds.

As an example of how a stopped flow apparatus may work, consider a simple irreversible reaction involving the binding of a ligand A to a receptor B that can undergo

the reaction

$$\mathrm{A} + \mathrm{B} \xrightarrow{k} \mathrm{AB}. \tag{6.2}$$

The ligand A has a fluorophore attached to it, and the receptor B has a quencher, such that the progress in the reaction can be monitored by loss of fluorescence signal over time. Assuming complete quenching when A is bound, the fluorescence is

$$f = f_0\, c_\mathrm{A}, \tag{6.3}$$

where f_0 is a constant of proportionality between the concentration of A and the measured fluorescence. We will call a function that maps chemical concentrations to a measured quantity a **readout function** (my term; I do not know of an "official" term for this). We have previously worked out that if the inlet concentrations of A and B are equal such that $c_\mathrm{A}^0 = c_\mathrm{B}^0 = c_0$,

$$c_\mathrm{A}(t) = \frac{c_0}{1 + c_0\, k\, t}. \tag{6.4}$$

Thus,

$$f(t) = \frac{f_0\, c_0}{1 + c_0\, k\, t} \tag{6.5}$$

Since c_0 is known f_0 and k can be found by performing a regression using this equation on the decay of fluorescence in the sample chamber over time.

6.3 Relaxation experiments

Relaxation experiments are a commonly used method for assessing reaction rates of reversible reactions. In a relaxation experiment, a reaction mixture is allowed to come to a steady state (where all time derivatives vanish). Then, conditions are suddenly and subtly changed and the dynamics observed. This usually involves a jump in temperature, pH, or pressure. Temperature or pH jumps can be made very rapidly using pulsed lasers to raise the temperature or photolyze absorbing molecules, respectively. In any case, the steady state (or equilibrium as we will soon learn about) shifts, since rate constants are in general dependent on temperature, pH, etc.

As an example, consider the reversible binding of two species A and B,

$$\mathrm{A} + \mathrm{B} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{AB}\,. \tag{6.6}$$

The dynamical equations for this reaction are

$$\frac{\mathrm{d}c_\mathrm{AB}}{\mathrm{d}t} = -\frac{\mathrm{d}c_\mathrm{A}}{\mathrm{d}t} = -\frac{\mathrm{d}c_\mathrm{B}}{\mathrm{d}t} = k_1\, c_\mathrm{A}\, c_\mathrm{B} - k_{-1}\, c_\mathrm{AB}. \tag{6.7}$$

Alternatively, we can write a single differential equation in terms of the extent of reaction, $\phi = c_{AB} - c_{AB}^0$ as

$$\frac{d\phi}{dt} = k_1(c_A^0 - \phi)(c_B^0 - \phi) - k_{-1}(c_{AB}^0 + \phi). \tag{6.8}$$

It is useful here to employ the fractional conversion ξ (see section 3.4.1), which in this case is the extent of reaction divided by the total number of particles of the limiting reagent. Assuming A is limiting such that $c_A^0 \leq c_B^0$,

$$\xi = \frac{\Phi}{N_A^0} = \phi / c_A^0. \tag{6.9}$$

Dividing the differential equation by c_A^0, and assuming $c_{AB}^0 = 0$, we have

$$\frac{d\xi}{dt} = k_1 c_A^0 (1 - \xi)\left(\frac{c_B^0}{c_A^0} - \xi\right) - k_{-1}\,\xi. \tag{6.10}$$

We can show that for any set of reversible reactions in a closed system (one in which no material flows in or out) with dynamics governed by mass action that a steady state exists and is unique.[8] We will denote the steady state fractional conversion as ξ_{ss}. It is not important to find it for the discussion here, but it can be solved to be

$$\xi_{ss} = \frac{1}{2}\left(1 + \frac{c_B^0}{c_A^0} + \frac{k_{-1}}{k_1 c_A^0} - \sqrt{\left(1 + \frac{c_B^0}{c_A^0} + \frac{k_{-1}}{k_1 c_A^0}\right)^2 - 4\,\frac{c_B^0}{c_A^0}}\right). \tag{6.11}$$

Now, let's say we quickly make a perturbation, say by a temperature jump, such that the steady state shifts. Let us assume that immediately after the perturbation the new extent of reaction is $\xi_{ss} + \delta\xi$, where $\delta\xi$ is a small perturbation. After the temperature jump, the reaction mixture will relax back to steady state according to the above differential equation, or

$$\frac{d(\xi_{ss} + \delta\xi)}{dt} = k_1 c_A^0 (1 - \xi_{ss} - \delta\xi)\left(\frac{c_B^0}{c_A^0} - \xi_{ss} - \delta\xi\right) - k_{-1}(\xi_{ss} + \delta\xi). \tag{6.12}$$

We can group terms as

$$\begin{aligned}\left[\frac{d\xi_{ss}}{dt}\right] + \frac{d\delta\xi}{dt} = &\left[k_1 c_A^0 (1 - \xi_{ss})\left(\frac{c_B^0}{c_A^0} - \xi_{ss}\right) - k_{-1}\,\xi_{ss}\right] \\ &- k_1 c_A^0 \left(\left(1 + \frac{c_B^0}{c_A^0} - 2\xi_{ss}\right)\delta\xi - (\delta\xi)^2\right) - k_{-1}\,\delta\xi\end{aligned} \tag{6.13}$$

[8] We will prove this when we talk about equilibrium in the thermodynamics section of the course.

The bracketed terms are zero by the definition of the steady state. Further, if the perturbation is small, the $(\delta\xi)^2$ is negligible compared to those linear in $\delta\xi$. Thus, we have

$$\frac{\mathrm{d}\,\delta\xi}{\mathrm{d}t} = -\left(k_1\, c_{\mathrm{A}}^0 \left(1 + \frac{c_{\mathrm{B}}^0}{c_{\mathrm{A}}^0} - 2\,\xi_{\mathrm{ss}}\right) + k_{-1}\right)\ \delta\xi\,. \tag{6.14}$$

This is a first order linear differential equation with solution

$$\delta\xi(t) = \delta\xi_0\, \mathrm{e}^{-t/\tau}, \tag{6.15}$$

with the relaxation time τ given by

$$\tau = \frac{1}{k_1\, c_{\mathrm{A}}^0 \left(1 + \frac{c_{\mathrm{B}}^0}{c_{\mathrm{A}}^0} - 2\,\xi_{\mathrm{ss}}\right) + k_{-1}}. \tag{6.16}$$

It is traditional to write this relaxation time not in terms of the steady state fractional conversion, but in terms of the steady state concentrations, $c_{\mathrm{A}}^{\mathrm{ss}} = c_{\mathrm{A}}^0(1 - \xi_{\mathrm{ss}})$ and $c_{\mathrm{B}}^{\mathrm{ss}} = c_{\mathrm{A}}^0(1 - \xi_{\mathrm{ss}})$.

$$\tau = \frac{1}{k_1\,(c_{\mathrm{A}}^{\mathrm{ss}} + c_{\mathrm{B}}^{\mathrm{ss}}) + k_{-1}}. \tag{6.17}$$

To determine the rate constants k_1 and k_{-1}, one can perform experiments for various concentrations of reactants, thereby giving different steady state concentrations. For each relaxation experiment, the parameter τ is determined. Then, one can use the above expression in a regression to find the values of k_{-1} and k_1.

7 Bioreactors

Most of the important biochemistry done on the planet is done inside little reactors called cells. However, bioengineers, build reactors for industrial processes to harness the extraordinary catalytic power of enzymes. Sometimes those reactors are full of little microbial reactors! Finally, as we saw in the last lesson, reactors like stop flow apparatuses can be used to study reaction kinetics using purified proteins and substrates. In this lesson, we will investigate various types of reactors and how we can model the dynamics of chemical reactions happening in them.

7.1 Material balances

At the heart of our study of reactors is a **material balance**, which is an equation that keeps track of all of the molecules of a given kind. The equation is simple and intuitive; it is really just accounting.

$$\text{accumulation} = \text{input} - \text{output} + \text{net generation by chemical reaction}. \quad (7.1)$$

We have learned how you use mass action to write rates of generation by chemical reaction. The new wrinkle is specifying the input and outputs for reactors that allow them. To start, though, we will consider a reactor with no input or output.

7.1.1 Batch reactors

Perhaps the most common vessel is a **batch reactor**. In a batch reactor, reactants are added, the reaction mixture is mixed, and then the reaction proceeds. When the reaction is complete, the reactor is emptied. So, input and output are both zero. Defining r_i to be the rate of chemical reaction i in units of concentration per time, we can write the material balance as

$$\underbrace{\frac{\mathrm{d}N_j}{\mathrm{d}t}}_{\text{accumulation}} = \underbrace{0}_{\text{input}} - \underbrace{0}_{\text{output}} + \underbrace{\sum_i \nu_{ij}\, r_i\, V}_{\text{net gen rxn}}, \quad (7.2)$$

where V is the volume of the reaction mixture in the reactor and N_j is the number of molecules of species j. Note r_i varies in time. Note also that in general the volume can vary in time, since the density can change as reactions go forward. Exothermicity, for example, can lead to temperature changes that change the density. For many of the reactions we consider in class, we will assume that the reactor is isothermal and that the reaction mixture has constant density. Note that we do also assume the reaction volume is **well-mixed**, meaning that there are no areas of the reactor that are

enriched in any particular species. There is no explicit spatial dependence in the material balance for the batch reactor, nor has there been thus far in class. Toward the end of the class, when we discuss diffusion, we will consider spatial variation, but for now, we will always make a well-mixed assumption. As a result, we will draw a batch reactor like in Fig. 5a, containing a propeller indicating that the fluid is well-mixed.

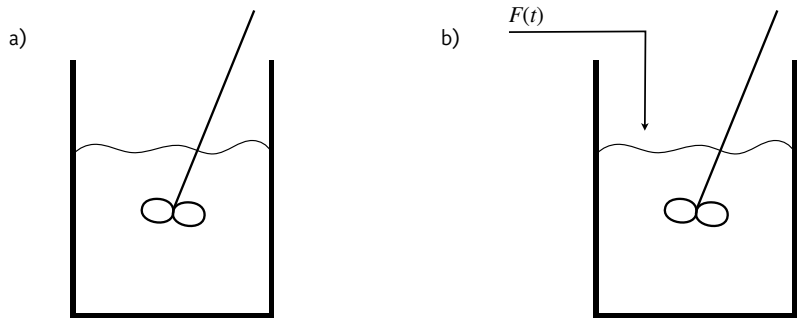


Figure 5: a) A batch reactor. b) A semibatch reactor. The volumetric inlet flow is $F(t)$, and there is no outlet flow.

When the volume is constant, we can conveniently write the material balance as

$$\frac{\mathrm{d}c_j}{\mathrm{d}t} = \sum_i \nu_{ij} r_i, \tag{7.3}$$

which is the same expression we have been using thus far for all of our governing equations. So, we have really been picturing all of our chemical processes thus far happening in a batch reactor, in our minds being a cell, a cuvette, a test tube, the sample chamber of a stopped flow device, etc.

7.1.2 Semibatch reactors

A **semibatch reactor**, shown in Fig. 5b, is like a batch reactor in that there is no effluent, but does have an inlet flow, called a **feed**. The **volumetric flow rate** of the inlet if $F(t)$ and has units of volume per time. We assume the feed has concentration c_j^0 of species j. Then, the material balance is

$$\underbrace{\frac{\mathrm{d}N_j}{\mathrm{d}t}}_{\text{accumulation}} = \underbrace{F\,c_j^0}_{\text{input}} - \underbrace{0}_{\text{output}} + \underbrace{\sum_i \nu_{ij}\, r_i\, V}_{\text{net gen rxn}}, \tag{7.4}$$

where

$$V(t) = V_0 + \int_0^t \mathrm{d}t'\, F(t'), \tag{7.5}$$

with V_0 being the volume of reaction mixture at time $t = 0$.

Semibatch reactors are often set up to contain some reagents initially, and then the feed contains the rest of the reagents necessary to start and continue the reaction. This is useful, for example, to slowly add in metabolites to maintain growth of microbes.

As an example of the dynamics we would observe in a semibatch reactor, consider the irreversible reaction

$$\mathrm{A} + \mathrm{B} \xrightarrow{k} \mathrm{AB}. \tag{7.6}$$

Initially, the semibatch reactor has no B in it, but has concentration c_{A}^0. The feed has no A in it, but has concentration c_{B}^0. We will assume a constant feed from time $t = 0$ until time t_{end}, at which time the contents of the reactor are emptied and the product AB is separated. So, the feed is

$$F(t) = \begin{cases} F_0 & 0 \le t \le t_{\mathrm{end}} \\ 0 & \text{otherwise.} \end{cases} \tag{7.7}$$

We can then write the volume as

$$V(t) = V_0 + \int_0^t \mathrm{d}t'\, F(t') = V_0 + F_0\, t, \tag{7.8}$$

where it is understood that $0 \le t \le t_{\mathrm{end}}$. The material balance for species A is then

$$\frac{\mathrm{d}N_{\mathrm{A}}}{\mathrm{d}t} = -k\, c_{\mathrm{A}}\, c_{\mathrm{B}}\, V = -k\, \frac{N_{\mathrm{A}}\, N_{\mathrm{B}}}{V^2}\, V = -k\, \frac{N_{\mathrm{A}}\, N_{\mathrm{B}}}{V_0 + F_0\, t}, \tag{7.9}$$

and that for species B is

$$\frac{\mathrm{d}N_{\mathrm{B}}}{\mathrm{d}t} = F_0\, c_{\mathrm{B}}^0 - k\, c_{\mathrm{A}}\, c_{\mathrm{B}}\, V = F_0\, c_{\mathrm{B}}^0 - k\, \frac{N_{\mathrm{A}}\, N_{\mathrm{B}}}{V_0 + F_0\, t}. \tag{7.10}$$

This system of nonlinear equations does not permit an analytical solution, so we can solve it numerically. We are well-served, as usual, to nondimensionalize. Although N_{A} and N_{B} are already dimensionless, but it is useful to redefine them in order to eliminate parameters. We will define constants n_{A}, n_{B}, and τ such that

$$N_{\mathrm{A}} = n_{\mathrm{A}}\, \tilde{N}_{\mathrm{A}}, \tag{7.11}$$

$$N_{\mathrm{B}} = n_{\mathrm{B}}\, \tilde{N}_{\mathrm{B}}, \tag{7.12}$$

$$t = \tau\, \tilde{t}. \tag{7.13}$$

We seek convenient expressions for these constants in our nondimensionalization procedure. Inserting the above expressions into the dynamical equations yields

$$\frac{n_{\mathrm{A}}}{\tau}\, \frac{\mathrm{d}\tilde{N}_{\mathrm{A}}}{\mathrm{d}\tilde{t}} = -\frac{k\, n_{\mathrm{A}}\, n_{\mathrm{B}}}{V_0}\, \frac{\tilde{N}_{\mathrm{A}}\, \tilde{N}_{\mathrm{B}}}{1 + \frac{F_0}{V_0}\, \tau\, \tilde{t}}, \tag{7.14}$$

$$\frac{n_B}{\tau}\frac{d\tilde{N}_B}{d\tilde{t}} = F_0\, c_B^0 - \frac{k\, n_A\, n_B}{V_0}\frac{\tilde{N}_A\,\tilde{N}_B}{1+\frac{F_0}{V_0}\,\tau\,\tilde{t}}, \tag{7.15}$$

where we have factored a V_0 out of the denominators of the chemical reaction term. Immediately upon reviewing these equations, we see that

$$\tau = \frac{V_0}{F_0} \tag{7.16}$$

is a convenient choice. This is a semibatch analog to the **space time** that we will learn about momentarily when we talk about continuously stirred tank reactors. Using the space time for τ and rearranging yields

$$\frac{d\tilde{N}_A}{d\tilde{t}} = -\frac{k\, n_B}{F_0}\frac{\tilde{N}_A\,\tilde{N}_B}{1+\tilde{t}}, \tag{7.17}$$

$$\frac{d\tilde{N}_B}{d\tilde{t}} = \frac{c_B^0\, V_0}{n_B} - \frac{k\, n_A}{F_0}\frac{\tilde{N}_A\,\tilde{N}_B}{1+\tilde{t}}. \tag{7.18}$$

We see that if we choose $n_A = n_B = c_B^0\, V_0$, we get

$$\frac{d\tilde{N}_A}{d\tilde{t}} = -\kappa\,\frac{\tilde{N}_A\,\tilde{N}_B}{1+\tilde{t}}, \tag{7.19}$$

$$\frac{d\tilde{N}_B}{d\tilde{t}} = 1 - \kappa\,\frac{\tilde{N}_A\,\tilde{N}_B}{1+\tilde{t}}, \tag{7.20}$$

where we have a single dimensionless parameter,

$$\kappa = \frac{V_0}{F_0}\, k\, c_B^0. \tag{7.21}$$

This is a ratio of time scales, that of the space time to the time scale of the reaction.

The numerical solution of this system of equations is shown in Fig. 6. As B is fed into the reactor, A gets converted into AB, and the AB concentration grows. Eventually, the A is exhausted and extra feed just dilutes the reaction mixture.

7.2 Continuous flow stirred-tank reactors

Continuous flow stirred-tank reactors, called CSTRs for short, shown in Fig. 7, have both a feed and an outflow. These reactors see widespread use in industrial biological processes, but are also used in research instruments for studying reaction rates. They offer a convenient look into the dynamics of chemical reactions when operated at steady state, we will be evident when we consider the material balance for a CSTR.

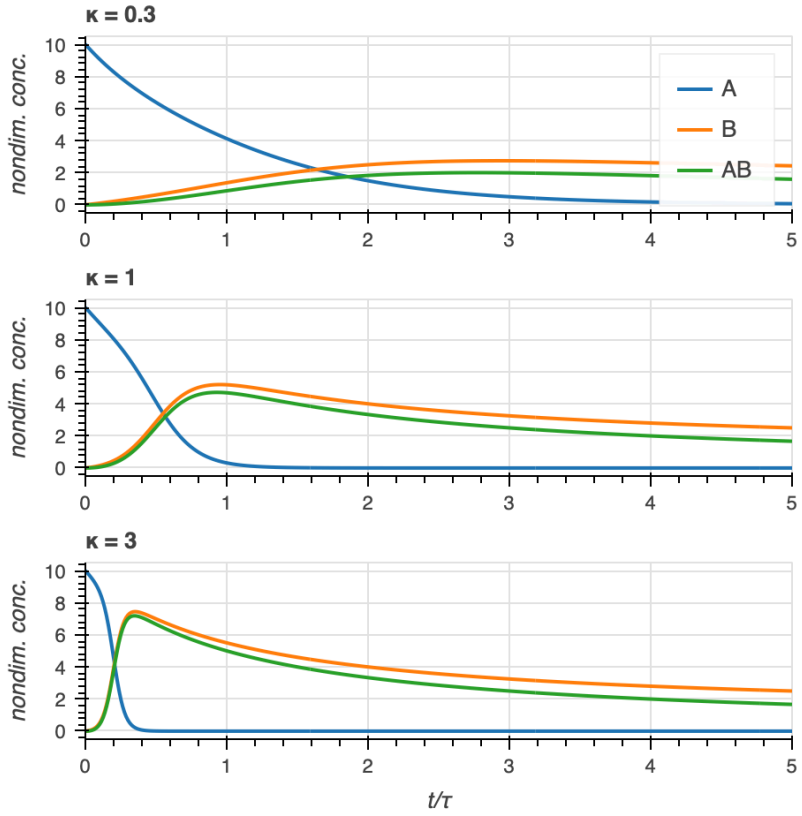


Figure 6: Numerical solution for the dynamics of the reaction A + B $\rightarrow$ AB in a semibatch reactor for various values of κ. The initial condition is $\tilde{N}_A(0) = 10$. The dimensionless concentration of species j is $\tilde{N}_j/(1+t)$, where $1+t$ is the dimensionless volume of the reaction mixture. This figure was generated with Listing 1.

The material balance now includes nonzero inputs, outputs, and net generation by chemical reaction. The material balance for species j is

$$\underbrace{\frac{\mathrm{d}N_j}{\mathrm{d}t}}_{\text{accumulation}} = \underbrace{F_{\text{in}}\, c_j^0}_{\text{input}} - \underbrace{F_{\text{out}}\, c_j}_{\text{output}} + \underbrace{\sum_i \nu_{ij}\, r_i\, V}_{\text{net gen rxn}}. \tag{7.22}$$

Note that V here is the volume of the reaction mixture, not the total volume of the reactor.

CSTRs are often operated at steady state, such that the accumulation term is zero. Again assuming constant density the volumetric flow rate into the CSTR must equal that out of the CSTR at steady state, such that $F_{\text{in}} = F_{\text{out}}$. Thus, at steady state,

$$0 = F\, c_j^0 - F\, c_j + \sum_i \nu_{ij}\, r_i\, V. \tag{7.23}$$

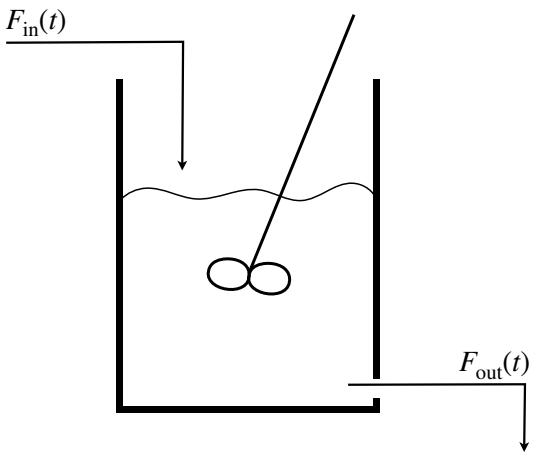


Figure 7: A continuous flow stirred-tank reactor (CSTR).

Assuming for a moment a single reaction, we can drop the sum over reactions and drop the i index, this is

$$0 = F\,c_j^0 - F\,c_j + \nu_j\,r\,V. \tag{7.24}$$

Rearranging,

$$r = \frac{c_j - c_j^0}{\nu_j\,\tau}, \tag{7.25}$$

where

$$\tau = \frac{V}{F} \tag{7.26}$$

is the **space time**. The inverse of the space time is called the **space velocity**. (Note that the space velocity is sometimes referred to as the **dilution rate**, as is the case in the Wittrup, Tidor, Hackel, and Sarkar book.) Evidently, with a CSTR, the rate of a reaction can be determined simply by operating at steady state and measuring the concentrations of the inlet and outlet along with the volumetric flow rate!

The space time is an important operational parameter of a CSTR. The volume is usually chosen based on how big a physical reactor is, and then the feed is chosen to achieve a desired space time. This is an important consideration because the fractional conversion for a chemical reaction, while independent of the volume of the reaction mixture for a batch reactor, *is* dependent on volume for a CSTR. The fractional conversion is *in*dependent of volume however, if the space time is the same. To make this explicit, consider a reaction proceeding in a constant-volume batch reactor.

$$\frac{\mathrm{d}c_j}{\mathrm{d}t} = \nu_j r. \tag{7.27}$$

Assuming species j is limiting, and noting that the fractional conversion is $\xi = 1 - c_j/c_j^0$, we can write the material balance instead as

$$-\frac{1}{\nu_j c_j^0}\frac{\mathrm{d}\xi}{\mathrm{d}t} = r. \tag{7.28}$$

There is clearly no volume dependence here, and that time necessary to achieve fractional conversion ξ is

$$t = c_j^0 \int_0^\xi \frac{\mathrm{d}\xi'}{r}. \tag{7.29}$$

Conversely, for a CSTR, we have

$$r = \frac{c_j - c_j^0}{\nu_j \tau} = -\frac{c_j^0}{\nu_j \tau}\,\xi. \tag{7.30}$$

Here, we see that τ is a function of ξ,

$$\tau = -\frac{c_j^0}{\nu_j r}\,\xi. \tag{7.31}$$

Finally, note that the space time is the average residence time of a single species in a CSTR. It is important to note that it is the *average* time and *not* how long any given molecule or microbe stays in a CSTR.

7.3 Chemostats

A **chemostat** is a bioreactor operating at steady state containing microbes that grow, divide, and usually produce a product of interest. The microbes and chemical species in the effluent are later separated and purified. So, a chemostat may be thought of as a CSTR where the "reaction" involved is microbial metabolism and growth.

We start by writing a material balance for microbes. Let x be the concentration of microbial cells. For microbes in ideal growth conditions, the growth is exponential with

$$\frac{\mathrm{d}x}{\mathrm{d}t} = kx, \tag{7.32}$$

where k is the growth rate. Then, operating at steady state, the chemostat material balance for microbes is

$$0 = Fx_0 - Fx + kxV. \tag{7.33}$$

Typically the feed has no microbes in it; reactors are usually seeded and then filled in a start-up process before operating at steady state. So, taking $x_0 = 0$, we find that the microbe concentration cancels from the material balance, giving

$$k = \frac{1}{\tau} = \frac{F}{V}, \tag{7.34}$$

demonstrating that the growth rate is equal to the space velocity. This is quite remarkable. By tuning the space time, which is entirely determined by the feed rate and the volume of the media/microbe mixture, the growth rate may be set.

Of course, this only works for nonzero k. We cannot have feed completely devoid of growth substrate for the microbes. In general, the growth rate will be dependent on the composition of the media, temperature, and other factors, so in order to achieve growth, the feed must be of the appropriate temperature and also have substrate in it to sustain growth. Let us then consider a steady state material balance on growth substrate, noting that the rate of substrate consumption is proportional to the rate of microbial growth. The constant of proportionality given by the **yield coefficient**. In general a yield coefficient is a ratio,

$$Y = \frac{\text{rate of production of a product}}{\text{rate of consumption of a feed}}. \tag{7.35}$$

The product could be anything of interest, such as microbes, waste, relevant chemical product, or even heat. Similarly the feed can be anything of interest. In this case, the product is microbes and the feed is substrate, such that

$$Y = \frac{\text{rate of microbial growth}}{\text{rate of consumption of substrate}}. \tag{7.36}$$

Denoting the substrate concentration as c and using the yield coefficient, the material balance for substrate is

$$0 = F\,c_0 - F\,c - \frac{1}{Y}\,k\,x\,V. \tag{7.37}$$

Noting that $k = 1/\tau = F/V$, we have

$$F(c_0 - c) = \frac{Fx}{Y}, \tag{7.38}$$

which is solved to give the steady state microbial concentration,

$$x = Y(c_0 - c). \tag{7.39}$$

While the yield is generally known for substrates and microbes under various conditions (≈ 0.5 for *E. coli* or *S. cerevisiae* fed with glucose), we need think about how to set c_0, F, and V to achieve a desired rate of growth, which usually is proprotional

to a production rate of product of interest. To do so, we need to be mindful that the growth rate k is a function of substrate concentration; $k = k(c)$. If a precise relationship between the growth rate and substrate concentration is known for a given set of conditions, we may use that. In lieu of that, we will use an expression proposed by Monod, and referred to as the **Monod equation**,

$$k(c) = k_{\max} \frac{c/K_S}{1 + c/K_S}. \tag{7.40}$$

This calls to mind the Langmuir isotherm and Michaelis-Menten kinetics. It is phenomenological and many growth rates do not follow the Monod expression, but it is still useful for conceptualizing design of bioreactors. Using this expression for the growth rate and recalling that the growth rate is given by the space velocity at steady state, we have

$$\tau = \frac{V}{F} = \frac{1}{k} = \frac{1}{k_{\max}} \frac{1 + c/K_s}{c/K_s} = \frac{1}{k_{\max}} \left(1 + \frac{K_s}{c}\right). \tag{7.41}$$

We can solve for the substrate concentration in terms of the space time,

$$c = \frac{K_s}{\tau k_{\max} - 1}. \tag{7.42}$$

Substituting this into the material balance of substrate (7.39),

$$x = Y\left(c_0 - \frac{K_s}{\tau k_{\max} - 1}\right) \tag{7.43}$$

This expression is not always positive. If

$$c_0 \leq \frac{K_s}{\tau k_{\max} - 1}, \tag{7.44}$$

the bacterial concentration is negative. Of course this is physically unrealizable; the resulting steady state is zero, meaning that all microbes flow out of the CSTR. In order to achieve a nonzero steady state microbial concentration, then, we must have a sufficiently large space time,

$$\tau > \tau_{\min} = \frac{1}{k_{\max}} \left(1 + \frac{K_s}{c_0}\right). \tag{7.45}$$

This means we need to operate with a large volume and small feed, lest we put the reactor in a **washout condition** in which the microbial population goes to zero.

With this knowledge, how should we set the operating conditions? If we want to optimize microbial growth rate (and therefore whatever product they are producing),

we want to find where $kx = x/\tau$ is maximal. Using our expression (7.43) for the bacterial concentration, we can find where x/τ is maximal by differentiating.

$$\begin{aligned}\frac{\mathrm{d}\,x/\tau}{\mathrm{d}\,\tau} &= \frac{\mathrm{d}}{\mathrm{d}\,\tau}\,\frac{Y}{\tau}\left(c_0 - \frac{K_\mathrm{s}}{\tau k_\mathrm{max} - 1}\right) \\ &= -\frac{Y}{\tau^2}\left(c_0 - \frac{K_\mathrm{s}}{\tau\, k_\mathrm{max} - 1}\right) + \frac{Y}{\tau}\,\frac{k_\mathrm{max}\,K_\mathrm{s}}{(\tau\, k_\mathrm{max} - 1)^2} = 0. \end{aligned} \tag{7.46}$$

Solving for τ, which involves some algebraic grunge, yields

$$\tau_\mathrm{opt} = \frac{1}{k_\mathrm{max}}\left(1 + \frac{K_\mathrm{s}}{c_0} + \sqrt{\frac{K_\mathrm{s}}{c_0}\left(1 + \frac{K_\mathrm{s}}{c_0}\right)}\right). \tag{7.47}$$

Typically the concentration of substrate in the feed is chosen such that $c_0 \gg K_\mathrm{s}$. (K_s for glucose is about 3 mg/L for *E. coli* and about 25 mg/mL for *S. cerevisiae*.) It is wise not to choose it to be too far above K_s, as solutions with high concentrations of sugar tend to be viscous and therefore more difficult to mix and aerate, but large enough that the microbial growth rate is near k_max. With $c_0 \gg K_\mathrm{s}$, the optimal space time we just calculated is very close to τ_min. This presents a problem because small variations in operating conditions could push the reactor into a washout condition. In practice, then, it would be wise to choose a space time to be a bit longer than τ_opt to about potentially disastrous washout conditions.

Part II

Thermodynamics

8 Mathematical preliminaries

As we shift our focus to thermodynamics, we need to lay some groundwork with some mathematical preliminaries.

8.1 Probability review

The mathematical scaffolding of statistical mechanics, which we will use to build up a thermodynamic theory, is the theory of probability. We will not formally define probability, but instead will propose a *working* definition[9] that is particularly useful ina statistical mechanical context, and then list some key features of probability.

8.1.1 Working definition of probability

Say we have N total outcomes, N_A of which are in category A. The probability of having an outcome in category A is

$$P(A) = \frac{N_A}{N}, \tag{8.1}$$

which is the fraction of all of the outcomes that are in category A. As an example, imagine that we roll a fair six sided die. Define A to be the set of outcomes where we roll an even number. In this case, $N = 6$ (each of the six sides of the die) and $N_A = 3$ (each of the sides of the die with an even number of pips), and

$$P(\text{even}) = \frac{3}{6} = \frac{1}{2}. \tag{8.2}$$

As another example, say we roll two dice. What is the probability of having the sum of the results be even? This is a bit more tricky, since there are six subcategories of even outcomes (2, 4, 6, 8, 10, and 12), but only five subcategories of odd outcomes (3, 5, 7, 9, and 11). So, careful counting is in order. In the table below, we list the (first die, second die) numbers to achieve each sum, and tally how many ways there are to achieve each sum.

[9] By working definition, I mean that it is not formal and not as fully applicable as a more formal definition of probability, but will suffice for our purposes.

sum of dice	ways to achieve	degeneracy
2	(1, 1)	1
3	(1, 2), (2, 1)	2
4	(1, 3), (2, 2), (3, 1)	3
5	(1, 4), (2, 3), (3, 2), (4, 1)	4
6	(1, 5), (2, 4), (3, 3), (4, 2), (5, 1)	5
7	(1, 6), (2, 5), (3, 4), (4, 3), (5, 2), (6, 1)	6
8	(2, 6), (3, 5), (4, 4), (5, 3), (6, 2)	5
9	(3, 6), (4, 5), (5, 4), (6, 3)	4
10	(4, 6), (5, 5), (4, 6)	3
11	(5, 6), (6, 5)	2
12	(6, 6)	1

Note that in the table we have used the term **degeneracy**, a term to mean the number of ways to achieve the outcomes in a given category. To compute the probability of rolling an even number, we sum up the degeneracies of the even sums, $N_A = 1 + 3 + 5 + 5 + 3 + 1 = 18$, and $N = 36$, giving $P(\text{even}) = 18/36 = 1/2$.

8.1.2 Properties of probability

Now that we have explored probability from some simple examples, we will sharpen our language a bit so that we can explore properties of probability.

- The **sample space** Ω is the set of all possible outcomes.
- An **event** is a subset of Ω. We colloquially used the term "category" for this concept before.
- Events A and B are **disjoint**, also called **mutually exclusive** if $A \cap B = \emptyset$. That is to say that two events are disjoint if they do not overlap at all in the sample space; they do not share any outcomes.

With these terms in hand, we can write some key properties of probability.

- The probability of any event is nonnegative; $P(A) \geq 0$.
- The probability of an outcome lying in the sample space is one, $P(\Omega) = 1$. This means that $P(A) + P(\text{not } A) = 1$, a result known as the **sum rule**. The follows that the probability of any event is also not greater than one, or $0 \leq P(A) \leq 1$.
- The probability of no outcome is zero, $P(\emptyset) = 0$.
- If $A_1, A_2, \ldots$ are disjoint, then

$$P\left(\bigcup_i A_i\right) = \sum_i P(A_i). \tag{8.3}$$

This means that probability is additive. The probability of observing an event in the union of disjoint events is the sum of the probabilities of those events.

Now, let's say that we are interested in event A happening *given* that event B happened. So, A is **conditioned** on B. We denote this **conditional probability** as $P(A \mid B)$. Given this notion of conditional probability, we can write the sum rule as

$$P(A \mid B) + P(\text{not } A \mid B) = 1, \tag{8.4}$$

for any B.

The **product rule** states that

$$P(A, B) = P(A \mid B)\, P(B), \tag{8.5}$$

where $P(A, B)$ is the probability of both A *and* B. (It could be, and often is, written as $P(A \cap B)$.) The product rule is also referred to as the **definition of conditional probability**.

Events A and B are said to be **independent**, if B has no bearing on A, such that

$$P(A \mid B) = P(A) \qquad (A \text{ and } B \text{ independent}). \tag{8.6}$$

As a result, the product rule gives, for independent A and B,

$$P(A, B) = P(A)P(B) \qquad (A \text{ and } B \text{ independent}). \tag{8.7}$$

This generalizes; if $A_1, A_2, \ldots$ are all independent of each other, then

$$P(A_1, A_2, \ldots) = \prod_i P(A_i) \qquad (\text{all } A_i \text{ independent}). \tag{8.8}$$

With these definitions and properties in hand, let's go back to the roll of two dice. With our sharper language, the event we are interested in is that the sum of the two dice is even. The sample space is all possible outcomes of the roll of two dice. The probability of a single die coming up even, as we already worked out is $1/2$. To get the probability of rolling an even sum with two dice, we can use conditional probability.

$$\begin{aligned} P(\text{sum even}) &= P(\text{second die even} \mid \text{first die even})P(\text{first die even}) \\ &\quad + P(\text{second die odd} \mid \text{first die odd})P(\text{first die odd}) \\ &= P(\text{second die even})P(\text{first die even}) \\ &\quad + P(\text{second die odd})P(\text{first die odd}), \end{aligned} \tag{8.9}$$

where we have dropped the conditioning because the result of the first die has no bearing on the result of the second die; they are independent. Thus, we have

$$P(\text{sum even}) = P(\text{second die even})P(\text{first die even})$$

$$+ P(\text{second die odd})P(\text{first die odd})$$

$$= \frac{1}{2}\cdot\frac{1}{2} + \frac{1}{2}\cdot\frac{1}{2} = \frac{1}{2}. \tag{8.10}$$

This was a more direct way to the same result we got by brute force counting.

8.1.3 Combinatorics and counting our way to probabilities

Working out probabilities often amounts to finding ways to count outcomes, which often involves working out the degeneracies of events, and then using some of the properties of probability to simplify. Let's consider a couple more examples.

Say I flip a fair coin N times. What is the probability of getting n heads? One way to get n heads is to have the first n flips land heads and the remaining $N-n$ land tails. Each flip is independent, so

$$P(\text{first } n \text{ heads}) = [P(\text{flip heads})]^n [P(\text{flip tails})]^{N-n} = \left(\frac{1}{2}\right)^n \left(\frac{1}{2}\right)^{N-n}. \tag{8.11}$$

But there are many more ways to get n heads. The degeneracy is the number of ways we can arrange the n heads flips across the N flips. Stated equivalently, we need to compute the number of way we can choose n out of N flips to be heads. We can use an useful result from combinatorics, which says that the number of ways to choose n out of N objects is

$$\binom{N}{n} = \frac{N!}{n!(N-n)!}, \tag{8.12}$$

pronounced "big N choose little n." So, the probability of flipping n out of N heads is

$$P(n) = \binom{N}{n} \left(\frac{1}{2}\right)^n \left(\frac{1}{2}\right)^{N-n}. \tag{8.13}$$

As another example, imagine we have an Eppendorf tube with a biomolecule in it. We divide the volume of the tube into little boxes such that each box can fit either a solvent molecule or our biomolecule. (We will neglect the obvious size difference here.) If we have N biomolecules and N_s solvent molecules, we have $N_s + N$ little boxes. If we are interested in the number of ways to distribute the biomolecules in space, we can compute the number of ways to choose N out of $N_s + N$ boxes.

$$\binom{N_s+N}{N} = \frac{(N_s+N)!}{N_s!N!}. \tag{8.14}$$

If we are interested in the probability that all of the biomolecules are at the bottom of the tube, assuming all configurations are equally probable (i.e., we are not operating a centrifuge or anything like that) we have the single configuration where the molecules are at the bottom, giving,

$$P(\text{all at the bottom}) = \frac{1}{\binom{N_s+N}{N}}, \tag{8.15}$$

and incredibly small number!

8.1.4 Probability distributions

Consider again the example of the probability of getting n head in N coin flips. We were able to write down

$$P(n) = \binom{N}{n} \left(\frac{1}{2}\right)^n \left(\frac{1}{2}\right)^{N-n}. \tag{8.16}$$

We did this because we knew that the probability that a given coin flip would land heads was one-half. We could have a biased coin, where the probability of heads is different from one-half. Let us call the probability of heads θ. In that case,

$$P(n) = \binom{N}{n} \theta^n \, (1-\theta)^{N-n}. \tag{8.17}$$

As we look at the above expression, we see that it is valid for *any* integer n with $0 \leq n \leq N$. This constitutes a **probability mass function**, or PMF, for the discrete outcomes n. We often write it as $P(n; N, \theta)$, since the probability is parametrized by N and θ.

The probability mass function is a feature of the **Binomial distribution**, and in fact uniquely defines it. We could otherwise define the Binomial distribution by another one of its features, its **cumulative distribution function**, or CDF,

$$F(n) = \sum_{n'=0}^{n} P(n'), \tag{8.18}$$

which gives the probability of flipping n or less heads in N flips.

Since the sample space for number of heads in n flips is exactly the integers n between zero and N, we have, by the sum rule,

$$\sum_{n=0}^{N} P(n) = \sum_{n=0}^{N} \binom{N}{n} \theta^n \, (1-\theta)^{N-n} = 1, \tag{8.19}$$

which indeed is the case.[10]

[10] That the Binomial probability mass function is normalized follows from the Binomial Theorem, which says that $\sum_n \binom{N}{n} a^n b^{N-n} = (a+b)^N$. For the Binomial probability mass function, we have $a = \theta$ and $b = 1 - \theta$ such that $a + b = 1$.

8.1.5 Moments of distributions

We may compute **expectation values**, or simply expectations, from probability distributions. The expectation of a function $h(x)$ of a probability distribution with PMF $P(x)$ is denoted $\langle h(x)\rangle_P$, and is given by

$$\langle h(x)\rangle_P = \sum_x h(x)\,P(x), \tag{8.20}$$

where the subscript P denotes that the expectation is computed over the probability distribution defined by P. This subscript is often dropped when the distribution over which the expectation is to be calculated is unambiguous from context.

The expectation for $h(x) = x$ is referred to as the **mean**, and is computed as

$$\langle x\rangle = \sum_x xP(x). \tag{8.21}$$

Another common expectation is the **variance**, which is the average square distance of values from the mean, such that $h(x) = (x - \langle x\rangle)^2$.

$$\langle (x - \langle x\rangle)^2\rangle = \sum_x (x - \langle x\rangle)^2 P(x). \tag{8.22}$$

Note that the variance may also be expressed as[11]

$$\langle (x - \langle x\rangle)^2\rangle = \langle x^2\rangle - \langle x\rangle^2. \tag{8.23}$$

As an example, we will compute the mean and variance for the Binomial distribution. First, the mean is calculated as

$$\begin{aligned}
\langle n\rangle &= \sum_{n=0}^{N} n\,\frac{N!}{(N-n)!n!}\,\theta^n(1-\theta)^{N-n} \\
&= \sum_{n=1}^{N} \frac{N!}{(N-n)!(n-1)!}\,\theta^n(1-\theta)^{N-n} \\
&= N\theta \sum_{n=1}^{N} \frac{(N-1)!}{(N-n)!(n-1)!}\,\theta^{n-1}(1-\theta)^{N-n}.
\end{aligned} \tag{8.24}$$

We make the substitutions $m = n - 1$ and $M = N - 1$ to get

$$\langle n\rangle = N\theta \sum_{m=0}^{M} \frac{M!}{(M-m)!m!}\,\theta^m(1-\theta)^{M-m} = N\theta, \tag{8.25}$$

[11] Can you derive this?

where the sum evaluates to unity because the Binomial distribution is normalized. To compute the variance, it is easier to compute

$$\begin{aligned}
\langle n(n-1)\rangle &= \sum_{n=0}^{N} n(n-1)\,\frac{N!}{(N-n)!n!}\,\theta^n(1-\theta)^{N-n} \\
&= \sum_{n=2}^{N} \frac{N!}{(N-n)!(n-2)!}\,\theta^n(1-\theta)^{N-n} \\
&= N(N-1)\,\theta^2 \sum_{n=2}^{N} \frac{(N-2)!}{(N-n)!(n-2)!}\,\theta^{n-2}(1-\theta)^{N-n} \\
&= N(N-1)\,\theta^2 \sum_{m=0}^{M} \frac{M!}{(M-m)!m!}\,\theta^m(1-\theta)^{M-m} \\
&= N(N-1)\,\theta^2, \qquad (8.26)
\end{aligned}$$

where this time we have made the substitutions $m = n-2$ and $M = N-2$. Thus, we have

$$\begin{aligned}
&\langle n(n-1)\rangle = \langle n^2\rangle - \langle n\rangle = N(N-1)\,\theta^2 \\
&\Rightarrow\ \langle n^2\rangle = N\theta(1-\theta+N\theta). \qquad (8.27)
\end{aligned}$$

Therefore, the variance is

$$\sigma^2 = \langle n^2\rangle - \langle n\rangle^2 = N\theta(1-\theta). \qquad (8.28)$$

8.1.6 Continuous distributions

In the previous examples, the variables were **discrete**; n took only integer values. If we have a continuous variable, instead of a probability mass function, we define a **probability density function**, $f(x)$, such that the probability of having x between x_1 and x_2 is

$$P(x_1 \le x \le x_2) = \int_{x_1}^{x_2} \mathrm{d}x f(x). \qquad (8.29)$$

Analogous definitions for expectations exist, with sums replaced by integrals.

$$\langle h(x)\rangle = \int \mathrm{d}x\, h(x) f(x), \qquad (8.30)$$

where the integral is a definite integral over the entire domain of x.

We conclude our probability review here.

8.2 Total differentials

Say we have a function of two variables, x and y, but x and y may themselves be functions of other variables, including, say, t. Then, but the chain rule, the **total derivative** of f with respect to t is

$$\frac{\mathrm{d}f}{\mathrm{d}t} = \frac{\partial f}{\partial x}\frac{\partial x}{\partial t} + \frac{\partial f}{\partial y}\frac{\partial y}{\partial t}. \tag{8.31}$$

We do not need to necessarily differentiate with respect to t, and can instead define a total differential element, called a **total differential**, $\mathrm{d}f$, as

$$\mathrm{d}f = \frac{\partial f}{\partial x}\,\mathrm{d}x + \frac{\partial f}{\partial y}\,\mathrm{d}y. \tag{8.32}$$

We will often use total differentials to define **thermodynamic potentials**.

8.3 Legendre transforms

Say we have a differentiable function $y(x)$ that gives a value y for each value of x. We could define the function as a set of (x, y) pairs. Now, for any point (x, y), we can define a slope

$$m(x) = \frac{\mathrm{d}y}{\mathrm{d}x} \tag{8.33}$$

of a line that is tangent to the curve $y(x)$. For each point x, the tangent line has an intercept $b(x)$ given by

$$b(x) = y(x) - m(x)\,x. \tag{8.34}$$

The value y can be obtained by the slope and intercept for any given point x. Thus, a collection of slope and intercept pairs has the same information as a collection of (x, y) pairs.

Now, we can define the total differential

$$\mathrm{d}y = \frac{\partial y}{\partial x}\,\mathrm{d}x. \tag{8.35}$$

We can also define a total differential for the intercept,

$$\mathrm{d}b = \mathrm{d}(y - m(x)\,x) = \mathrm{d}y - m\,\mathrm{d}x - x\,\mathrm{d}m, \tag{8.36}$$

where we have used the chain rule. However, by equation (8.35), $\mathrm{d}y - m\,\mathrm{d}x = 0$, so

$$\mathrm{d}b = -x\,\mathrm{d}m. \tag{8.37}$$

So, if we want to switch from a total differential for $\mathrm{d}y$ to one for $\mathrm{d}b$, we define

$$b = y - \frac{\partial y}{\partial x}\,\mathrm{dx} = y - mx. \tag{8.38}$$

The result is $\mathrm{d}b = -x\,\mathrm{d}m$. This change of variables is an example of a **Legendre transform**.

This generalizes to multivariate functions. E.g., if we have

$$\mathrm{d}z = m_x\,\mathrm{d}x + m_y\,\mathrm{d}y, \tag{8.39}$$

we can define $w = z - m_y y$ to get

$$\mathrm{d}w = m_x\,\mathrm{d}x - y\,\mathrm{d}m_y. \tag{8.40}$$

8.4 Homogeneous first order functions

A **homogeneous function of order** n is defined as a function such that

$$f(ax) = a^n(f(x)) \tag{8.41}$$

for nonzero scalar a. This generalizes to multivariate functions,

$$f(ax_1, ax_2, ax_3, \ldots) = a^n f(x_1, x_2, x_3, \ldots). \tag{8.42}$$

Assuming $n > 0$, differentiating the above relation with respect to a gives

$$\frac{\partial f(ax_1, ax_2, ax_3, \ldots)}{\partial a} = na^{n-1} f(x_1, x_2, x_3, \ldots). \tag{8.43}$$

We also have, for the total differential of $f(ax_1, ax_2, ax_3, \ldots)$,

$$\mathrm{d}f(ax_1, ax_2, ax_3, \ldots) = \sum_i \frac{\partial f(ax_1, ax_2, ax_3, \ldots)}{\partial (ax_i)}\,\mathrm{d}(ax_i). \tag{8.44}$$

Therefore,

$$\begin{aligned}\frac{\partial f(ax_1, ax_2, ax_3, \ldots)}{\partial a} &= \sum_i \frac{\partial f(ax_1, ax_2, ax_3, \ldots)}{\partial (ax_i)}\,\frac{\partial (ax_i)}{\partial a} \\ &= \sum_i \frac{\partial f(ax_1, ax_2, ax_3, \ldots)}{\partial (ax_i)}\,x_i.\end{aligned} \tag{8.45}$$

Together, Equations 8.43 and 8.45 give

$$f(x_1, x_2, x_3, \ldots) = \frac{1}{na^{n-1}} \sum_i \frac{\partial f(ax_1, ax_2, ax_3, \ldots)}{\partial (ax_i)}\,x_i. \tag{8.46}$$

This has to hold for any a. If we take $a = 1$, the result is **Euler's theorem for homogeneous functions**,

$$f(x_1, x_2, x_3, \ldots) = \frac{1}{n} \sum_i \frac{\partial f(x_1, x_2, x_3, \ldots)}{\partial (x_i)} x_i. \tag{8.47}$$

For a first-order homogeneous function, this is

$$f(x_1, x_2, x_3, \ldots) = \sum_i \frac{\partial f(x_1, x_2, x_3, \ldots)}{\partial x_i} x_i. \tag{8.48}$$

We will see that in thermodynamics, we will find that the entropy and energy are homogeneous functions of first order in their parameters, called **extensive parameters**. We will also find that intensive parameters are homogeneous functions of zero order of their extensive parameters. (There are the so-called equations of state.)

8.5 Method of Lagrange multipliers

We will very soon encounter the following optimization problem. We wish to find the maximum or minimum (generically, extremum) of a function $f(\mathbf{x})$, where f is scalar-valued and x may be vector-valued, subject to the constraint $c(\mathbf{x}) = 0$. That is, we want to find the value of $\mathbf{x}$ such that $f(\mathbf{x})$ is maximal or minimal, but we restrict ourselves only to values of $\mathbf{x}$ that satisfies $c(\mathbf{x}) = 0$.

It helps to consider a concrete example. Say I want to find the maximum of

$$f(\mathbf{x}) = f(x, y) = a_0 - (x - a_1)^2 - (y - a_2)^2. \tag{8.49}$$

The unconstrained maximum is clearly at $x = a_1$ and $y = a_2$. But, let's say we enforce that $x = y$ as a constraint. In this case,

$$c(\mathbf{x}) = c(x, y) = x - y. \tag{8.50}$$

With the constraint, the problem is more difficult. One approach is to simply substitute $y = x$ into the expression for $f(x, y)$ and find the maximizer of $f(x, x)$.

$$f(x, x) = a_0 - (x - a_1)^2 - (x - a_2)^2. \tag{8.51}$$

We now differentiate $f(x, x)$ with respect to x and set the result equal to zero to find the maximizer.

$$\frac{\mathrm{d}f}{\mathrm{d}x} = -2(x - a_1) - 2(x - a_2) = -4x + 2(a_1 + a_2) = 0, \tag{8.52}$$

which is readily solved to give

$$x^* = \frac{a_1 + a_2}{2}, \tag{8.53}$$

where the star superscript is used to denote an optimum.

This is all fine and good, but what if we cannot write x as a simple function of y, as we could do in this case? This often happens when we have many variables. This is where the **method of Lagrange multipliers** is useful. Recalling again that $f(x, y)$ is a function of x and y, we write, using the total derivative of $f(x, y)$ with respect to x, which must be zero at an extremum,

$$\frac{\mathrm{d}f}{\mathrm{d}x} = \frac{\partial f}{\partial x}\frac{\partial x}{\partial x} + \frac{\partial f}{\partial y}\frac{\partial y}{\partial x} = \frac{\partial f}{\partial x} + \frac{\partial f}{\partial y}\frac{\partial y}{\partial x} = 0. \tag{8.54}$$

In the case where there is no constraint, $\partial y/\partial x = 0$ and we get an extremum when the partial derivative of f with respect to x (and also y) vanishes. But $\partial y/\partial x$ can be nonzero when there are constraints.

We also can write the total derivative of the constraint, noting that it vanishes because $c(x, y) = 0$ by definition.

$$\frac{\mathrm{d}c}{\mathrm{d}x} = \frac{\partial c}{\partial x}\frac{\partial x}{\partial x} + \frac{\partial c}{\partial y}\frac{\partial y}{\partial x} = \frac{\partial c}{\partial x} + \frac{\partial c}{\partial y}\frac{\partial y}{\partial x} = 0. \tag{8.55}$$

From our expression for the total derivative of f, equation (8.54), we have

$$\frac{\partial y}{\partial x} = -\frac{\partial f/\partial x}{\partial f/\partial y}. \tag{8.56}$$

From our expression for the total derivative of f, equation (8.54), we have

$$\frac{\partial y}{\partial x} = -\frac{\partial c/\partial x}{\partial c/\partial y}. \tag{8.57}$$

Taken together, these two equations give

$$\frac{\partial f/\partial x}{\partial f/\partial y} = \frac{\partial c/\partial x}{\partial c/\partial y}. \tag{8.58}$$

We can rearrange to give

$$\frac{\partial f/\partial x}{\partial c/\partial x} = \frac{\partial f/\partial y}{\partial c/\partial y}. \tag{8.59}$$

This equation relates derivatives of different functions with respect to different variables. The only way this equality may hold if both the right hand side and left hand

side are equal to a constant. We call this constant $-\lambda$, where λ is called a **Lagrange multiplier**.

$$\frac{\partial f/\partial x}{\partial c/\partial x} = \frac{\partial f/\partial y}{\partial c/\partial y} = -\lambda\,. \tag{8.60}$$

We can equivalently write this equation as two equations,

$$\frac{\partial f}{\partial x} + \lambda\,\frac{\partial c}{\partial x} = 0, \tag{8.61}$$

$$\frac{\partial f}{\partial y} + \lambda\,\frac{\partial c}{\partial y} = 0. \tag{8.62}$$

These equations are the new conditions for optimality, including also the requirement that $c(x,y) = x - y = 0$. They followed from setting the total derivative of f to zero and enforcing equation (8.60), which follows from the constraints.

We can state these equations more succinctly by defining the **Lagrangian**, $\mathcal{L}(x, y, \lambda)$, as

$$\mathcal{L}(x, y, \lambda) = f(x,y) + \lambda\, c(x,y). \tag{8.63}$$

Then, the condition for optimality is

$$\frac{\partial \mathcal{L}}{\partial x} = \frac{\partial \mathcal{L}}{\partial y} = \frac{\partial \mathcal{L}}{\partial \lambda} = 0. \tag{8.64}$$

Let's try this out for our present optimization problem. The Lagrangian is

$$\mathcal{L} = a_0 - (x - a_1)^2 - (y - a_2)^2 + \lambda\,(x - y). \tag{8.65}$$

Then, for our optimality conditions we have

$$\frac{\partial \mathcal{L}}{\partial x} = -2(x - a_1) + \lambda = 0, \tag{8.66}$$

$$\frac{\partial \mathcal{L}}{\partial y} = -2(y - a_2) - \lambda = 0, \tag{8.67}$$

$$\frac{\partial \mathcal{L}}{\partial x} = x - y = 0. \tag{8.68}$$

These three equations are readily solved to give

$$x = y = \frac{a_1 + a_2}{2}, \tag{8.69}$$

$$\lambda = a_2 - a_1, \tag{8.70}$$

the same results as before. Check!

8.5.1 General formulation

In general, if we want to find $\mathbf{x}$ to optimize a differentiable function $f(\mathbf{x})$ subject to a set of differentiable equality constraints $c_1(\mathbf{x}) = 0$, $c_2(\mathbf{x}) = 0$, ..., then the Lagrangian is

$$\mathcal{L}(\mathbf{x}, \lambda) = f(\mathbf{x}) + \sum_i \lambda_i c_i(\mathbf{x}), \tag{8.71}$$

and the conditions

$$\nabla_{\mathbf{x}} \mathcal{L}(\mathbf{x}^*, \lambda^*) = 0, \tag{8.72}$$

$$\nabla_{\lambda} \mathcal{L}(\mathbf{x}^*, \lambda^*) = 0, \tag{8.73}$$

are necessary for x^* to be optimal. These conditions are referred to as the **Karush-Kuhn-Tucker conditions**, or KKT conditions for short.[12]

For completeness, I state here sufficient conditions for minimizers and maximizers. You may skip this paragraph and continue to the next section on convex optimization, where the sufficient conditions are a special case of the more general conditions I present here. A sufficient condition for $\mathbf{x}^*$ and λ^* to be constrained minimizers of f is that $\mathcal{L}$ is **positive definite** at $\mathbf{x}^*$ and λ^*, namely

$$\mathbf{w}^{\mathsf{T}} \cdot (\nabla^2_{\mathbf{xx}} \mathcal{L}) \cdot \mathbf{w} > 0 \tag{8.74}$$

for any nonzero $\mathbf{w}$. This is the multidimensional analog to the second derivative being positive at a minimum of a one-dimensional function. Similarly, for $\mathbf{x}^*$ and λ^* to be constrained maximizers of f, it is sufficient that the Lagrangian be negative definite.

8.5.2 Convex constrained optimization

When the function $f(\mathbf{x})$ we are trying to optimize is **convex** and constraints are also convex (or affine, which is the case with linear constraints), the optimization problem has some convenient structure. A function $f(\mathbf{x})$ is convex if for any $\mathbf{x}$ and $\mathbf{y}$ on the domain of f,

$$f(\theta\mathbf{x} + (1 - \theta)\mathbf{y}) \leq \theta f(\mathbf{x}) + (1 - \theta) f(\mathbf{y}). \tag{8.75}$$

This is a mathematical statement that the curve lies below any cord between two points of the function.

[12] The KKT conditions are more generally defined also for *in*equality constraints as well as the equality constraints we consider here. For considering only equality constraints, the KKT conditions we listed here are complete.

We can use a more convenient condition for convexity if $f(\mathbf{x})$ is twice differentiable. A twice differentiable function $f(\mathbf{x})$ is convex if and only if its Hessian

$$\mathsf{B} \equiv \nabla^2_{\mathbf{xx}} f = \begin{pmatrix} \frac{\partial^2 f}{\partial x_1^2} & \frac{\partial^2 f}{\partial x_1 \, \partial x_2} & \cdots \\ \frac{\partial^2 f}{\partial x_2 \, \partial x_1} & \frac{\partial^2 f}{\partial x_2^2} & \cdots \\ \vdots & \vdots & \ddots \end{pmatrix} \tag{8.76}$$

is positive definite. Recall that B is positive definite if and only if:

i) $\mathbf{w}^{\mathsf{T}} \cdot \mathsf{B} \cdot \mathbf{w} > 0$ for all nonzero $\mathbf{w}$,

ii) All eigenvalues are positive,

iii) All upper-left submatrices have positive determinants,

iv) There exists a matrix R with independent columns such that $\mathsf{B} = \mathsf{R}^{\mathsf{T}} \cdot \mathsf{R}$.

And here is a very useful result. If $f(\mathbf{x})$ is convex and the constraints $c_i(\mathbf{x})$ are also convex (or affine, which means that the $c_i(\mathbf{x})$ is a linear function of $\mathbf{x}$), then the KKT conditions are necessary *and* sufficient for $\mathbf{x}^*$ and λ^* to be a *global* minimum. Conversely, if $f(\mathbf{x})$ is **concave** (meaning that its Hessian is *negative* definite), as are the constraints (or they are affine), then the KKT conditions are necessary and sufficient for $\mathbf{x}^*$ and λ^* to be a global *maximum*.

We will use these results from convex constrained optimization to derive expressions for the probability distribution of states of biomolecular systems and to prove properties relating to the existance and uniqueness of equilibria.

9 Entropy and the Boltzmann distribution

In this lesson, we will introduce the concept of **entropy** and its maximization, ultimately deriving the **Boltzmann distribution**.

9.1 Motivation: A two-state model for a protein

Say we have a protein that can be in two configurations, which we will label as "active" and "inactive." We will refer to each configuration as a **state**, often referred to as a **microstate**. This protein has two states. We are interested in working out the probability p_{active} that the protein will be in the active state. The probability that it will be in the inactive state is $p_{\text{inactive}} = 1 - p_{\text{active}}$. The machinery of statistical mechanics allows us to do this.

Though we are motivated by this two-state model, we will develop an expression for p_i, where i is one of an arbitrarily many states, more generally for any system with an associated set of discrete states. The states are indexed by i, and each has an energy E_i associated with it. We will maximize **informational entropy** in our treatment, following E. T. Jaynes, *Phys. Rev.*, **106**, 620–630, 1957. The abstract of that paper very cleanly and clearly captures the notion of what we are trying to do here.

> Information theory provides a constructive criterion for setting up probability distributions on the basis of partial knowledge, and leads to a type of statistical inference which is called the maximum-entropy estimate. It is the least biased estimate possible on the given information; i.e., it is maximally noncommittal with regard to missing information. If one considers statistical mechanics as a form of statistical inference rather than as a physical theory, it is found that the usual computational rules, starting with the determination of the partition function, are an immediate consequence of the maximum-entropy principle. In the resulting "subjective statistical mechanics," the usual rules are thus justified independently of any physical argument, and in particular independently of experimental verification; whether or not the results agree with experiment, they still represent the best estimates that could have been made on the basis of the information available.
>
> It is concluded that statistical mechanics need not be regarded as a physical theory dependent for its validity on the truth of additional assumptions not contained in the laws of mechanics (such as ergodicity, metric transitivity, equal a priori probabilities, etc.). Furthermore, it is possible to maintain a sharp distinction between its physical and statistical aspects. The former consists only of the correct enumeration of the states

of a system and their properties; the latter is a straightforward example of statistical inference.

9.2 The Shannon entropy

The problem of specifying p_i is really open-ended. As Jaynes suggested, we can use maximum-entropy principles to derive an expression for p_i. The entropy he is talking about is the **Shannon entropy**, named after Claude Shannon, who published its mathematical form in 1948, also known as the **informational entropy**. Formally, informational entropy is the reduction in ignorance derived from learning an outcome. It might be easier to think about ignorance instead.

Say event i happens with probability p_i. If i is very probable and we observe it, we haven't learned much. For example, if we observe that the current pope is Catholic, we haven't learned much about popes. That is, we are still pretty ignorant about popes. But if i is very *im*probable and we observe it, we have learned a lot. If we observe a pig flying, we have truly learned something new about nature.

To codify this in mathematical terms, we might think that the information gained by observing event i should scale like $1/p_i$, since more rare events give higher information.

Now, say we observe two *independent* events, i and j. Since they are totally independent, the information garnered from observing both should be the sum of the information garnered from observing each. However, we know that the probability of observing both is $p_i p_j$. But

$$\frac{1}{p_i} + \frac{1}{p_j} \neq \frac{1}{p_i p_j}. \tag{9.1}$$

So, our current metric of information as $1/p_i$ does not satisfy this addability requirement. However,

$$\log \frac{1}{p_i} + \log \frac{1}{p_j} = \log \frac{1}{p_i p_j}. \tag{9.2}$$

So, we choose $\log(1/p_i) = -\log p_i$ as a measure of information. We are free to choose the base of the logarithm, and it is traditional to choose base 2. The units of information are then called **bits**.

Now, say we have a whole sample space of events. Then the *average* information we get from observing events (i.e., the level of surprise, the loss of ignorance) is

$$S[p] = -\sum_i p_i \log p_i. \tag{9.3}$$

This is called the **Shannon entropy** or **informational entropy**.

Let's look at the Shannon entropy another way. Say we know all of the p_i's, meaning that we know the probability distribution describing the phenomena of interest. How much knowledge do we know about what events we might observe? If the probability distribution is flat, not much. Conversely, if it is sharply peaked, we know a lot about what event we will observe. In the latter case, observing one event does not give us more information beyond what we already knew from the probabilities. So, the entropy $S[p]$ *is a measure of ignorance*. It tells us how uncertain or unbiased we are ahead of an observation.

I pause to note that we shortcutted our way into this definition of entropy by using some logic and the desire that independent events add. A more careful derivation was done in 1948 by Claude Shannon. He showed that the function we wrote for the entropy is the *only* function up to a positive multiplicative constant that satisfies three desiderata about a measure of ignorance.

1. Entropy is continuous in p_i.
2. If all p_i are equal, entropy is monotonically increasing in N, the number of events we could observe.
3. Entropy satisfies a composition law; grouping of events does not change the value of entropy.

The derivation is beautiful, but we will not go into it here. We will also discover in lecture and in homework that the entropy defined this way also has other important properties, such as extensivity and convexity.

Since the Shannon entropy is the only function that can serve as a measure of ignorance, and is defined up to a positive multiplicative constant, we will go ahead and include it and call it K such that

$$S[p] = -K \sum_i p_i \ln p_i. \tag{9.4}$$

If we want entropy in units of bits, we choose $K = 1/\ln 2$.

Finally, I note that going forward, it is understood that all p_i's satisfy $0 \leq p_i \leq 1$, as all probabilities must, and that $p_i \ln p_i \to 0$ as p_i tends toward zero. This is verified using L'Hôpital's rule,

$$\begin{aligned} \lim_{p_i \to 0} p_i \ln p_i = \lim_{p_i \to 0} p_i \ln_b p_i &= \lim_{p_i \to 0} \frac{\ln p_i}{1/p_i} \\ &= -\lim_{p_i \to 0} \frac{1/p_i}{1/p_i^2} = -\lim_{p_i \to 0} p_i = 0. \end{aligned} \tag{9.5}$$

9.2.1 An example of Shannon entropy and its maximization

Imagine flipping a possibly biased coin. There are two outcomes, heads, with probability p_h, and tails, with probability $1 - p_h$. The entropy in units of bits is

$$S = -\sum_i p_i \log_2 p_i = -p_h \log_2 p_h - (1 - p_h) \log_2 (1 - p_h). \tag{9.6}$$

So, if the coin is unbiased, then $p_h = 1/2$ and $S = 1$ bit. Now, let's say $p_h = (1 + \varepsilon)/2$, where $\varepsilon \in [-1, 1]$. That is, the coin is biased if ε is nonzero. Now, we have

$$\begin{aligned} S &= -\frac{1+\varepsilon}{2} \log_2 \frac{1+\varepsilon}{2} - \frac{1-\varepsilon}{2} \log_2 \frac{1-\varepsilon}{2} \\ &= -\frac{1}{2} \log_2 \frac{(1+\varepsilon)(1-\varepsilon)}{4} - \frac{\varepsilon}{2} \log_2 \frac{1+\varepsilon}{1-\varepsilon} \\ &= 1 - \log_2 (1 - \varepsilon^2) - \frac{\varepsilon}{2} \log_2 \frac{1+\varepsilon}{1-\varepsilon} \\ &= 1 - \log_2 (1 - \varepsilon^2) - \frac{|\varepsilon|}{2} \log_2 \frac{1+|\varepsilon|}{1-|\varepsilon|}. \end{aligned} \tag{9.7}$$

Looking at the three terms, we have a constant plus two monotonically decreasing functions of $|\varepsilon|$. Further, if $|\varepsilon| = 1$, we get $S = 0$. So, the maximal entropy is when ε, the bias of the coin, is zero. The entropy is minimal when $|\varepsilon| = 1$, which means that we know the outcome of the coin toss ahead of time. Thus, the maximal entropy probability distribution for a coin flip is the one that is unbiased.

Now, imagine that instead of flipping a fair coin (which has two sides), we roll a fair 8-sided die. The entropy associated with the probability distribution for the die is

$$S = -\sum_i p_i \log_2 p_i = -8 \left(\frac{1}{8} \log_2 \frac{1}{8} \right) = 3 \text{ bits}. \tag{9.8}$$

So, the entropy for a fair 8-sided die is greater than that of a fair coin. This makes sense; we are more ignorant as to the result we would expect from an 8-sided die than from a two-sided coin.

9.2.2 Insufficient reason and maximum entropy

Just a moment ago, we posited that the probability distribution corresponding to a *fair* eight-sided die has the maximal entropy. We can prove this by maximizing the

entropy. Note that maximizing the entropy assumed the *least* amount of information about the probability distribution. To generalize, consider an N-sided die. The entropy is

$$S[p] = -K \sum_{i=1}^{N} p_i \, \ln p_i. \tag{9.9}$$

To maximize the entropy, we *could* (but we shouldn't!) differentiate the entropy with respect to p_i and set the derivative equal to zero.

$$\frac{\partial S}{\partial p_j} = -K \frac{\partial}{\partial p_j} \sum\nolimits_{i=1}^{N} p_i \ln p_i = -K(1 + \ln p_j) = 0 \quad \Rightarrow \quad p_j = \mathrm{e}^{-1}. \tag{9.10}$$

I put this equation in gray because this is *not* what we should do! Clearly this cannot be right, since the probability distribution is not normalized, i.e., $\sum_i p_i \neq 1$.

We need to do a *constrained* maximization. Specifically, we need to impose the constraint that $\sum_i p_i = 1$, as is always the case. We can use the method of Lagrange multipliers, introducing the Lagrange multiplier α and defining the Lagrangian as

$$\mathcal{L}(p_i, \alpha) = -K \sum_{i=1}^{N} p_i \, \ln p_i + \alpha \left(1 - \sum_{i=1}^{N} p_i \right). \tag{9.11}$$

As you will show in your homework, the entropy is concave in the probabilities p_i, and the constraint is affine, so the KKT conditions are necessary and sufficient for the entropy to be maximized.

$$\frac{\partial \mathcal{L}}{\partial p_j} = 0 \ \ \forall j, \tag{9.12}$$

$$\frac{\partial \mathcal{L}}{\partial \alpha} = 0. \tag{9.13}$$

Evaluating the first equation of the KKT conditions,

$$\frac{\partial \mathcal{L}}{\partial p_j} = -K(1 + \ln p_j) - \alpha = 0 \ \ \forall j. \tag{9.14}$$

This gives

$$p_j = \mathrm{e}^{-1-\alpha/K} \ \ \forall j. \tag{9.15}$$

We see immediately that all probabilities are the same. The second KKT condition gives the normalization condition.

$$\frac{\partial \mathcal{L}}{\partial \alpha} = 1 - \sum_{i=1}^{N} p_i = 0 \ \Rightarrow \sum_{i=1}^{N} p_i = 1. \tag{9.16}$$

Thus, we have

$$\sum_{i=1}^{N} \mathrm{e}^{-1-\alpha/K} = N\mathrm{e}^{-1-\alpha/K} = 1, \tag{9.17}$$

which gives

$$p_j = \mathrm{e}^{-1-\alpha/K} = \frac{1}{N}. \tag{9.18}$$

Indeed, each side of an N-sided die has equal probability when entropy is maximized. This concept that maximal ignorance involves assignment of equal probability to all outcomes was put forward by Laplace as the **principle of insufficient reason** long before concepts of entropy were understood.

9.3 The Boltzmann distribution

Thinking back to our original motivating example of a two-state protein, the principle of insufficient reason suggests that we should assign equal probability to each state. This would be the maximally ignorant way to assign the probabilities, as it is the maximal entropy distribution, subject only to the constraint that the probabilities of the states sum to one. But we are not *completely* ignorant. We know that each state has associated with it an energy, which we shall denote as E_i. Since each state has an energy, the probability distribution should have an expectation value for the energy,

$$\langle E \rangle = \sum_i E_i p_i. \tag{9.19}$$

Thus, we have another constraint.

Moving again to the general case where there are many states, we can include the existence of an expectation value of energy as a new constraint. The Lagrangian for the now more-constrained maximization of entropy problem is

$$\begin{aligned}\mathcal{L}(p_i, \alpha, \beta) &= S + \alpha \left(1 - \sum_i p_i\right) + \beta \left(\langle E \rangle - \sum_i p_i E_i\right) \\ &= -K \sum_i p_i \ln p_i + \alpha \left(1 - \sum_i p_i\right) + \beta \left(\langle E \rangle - \sum_i p_i E_i\right),\end{aligned} \tag{9.20}$$

where we have introduced a second Lagrange multiplier, β, to enforce the expectation of the energy constraint. This constraint is again linear, so the KKT conditions are again necessary and sufficient to maximize the entropy.

$$\frac{\partial \mathcal{L}}{\partial p_j} = 0 \ \forall j, \tag{9.21}$$

$$\frac{\partial \mathcal{L}}{\partial \alpha} = 0, \tag{9.22}$$

$$\frac{\partial \mathcal{L}}{\partial \beta} = 0. \tag{9.23}$$

Considering the first KKT condition,

$$\frac{\partial \mathcal{L}}{\partial p_j} = -K(1 + \ln p_j) - \alpha - \beta E_j = 0 \ \ \forall j. \tag{9.24}$$

Solving for p_j gives

$$p_j = \mathrm{e}^{-1-\alpha/K}\, \mathrm{e}^{-\beta E_j/K}. \tag{9.25}$$

We can redefine constants such that $1 + \alpha/K \to \alpha$ and $\beta/K \to \beta$, giving a simpler expression

$$p_j = \mathrm{e}^{-\alpha}\, \mathrm{e}^{-\beta E_j}. \tag{9.26}$$

Now, using the normalization constraint, which follows from the KKT condition that $\partial \mathcal{L}/\partial \alpha = 0$, we have

$$\sum_i p_i = \mathrm{e}^{-\alpha} \sum_i \mathrm{e}^{-\beta E_i} = 1, \tag{9.27}$$

so that

$$\mathrm{e}^{\alpha} = \sum_i \mathrm{e}^{-\beta E_i} \equiv Z, \tag{9.28}$$

where we have defined the **partition function** Z. The second constraint coming from the last KKT condition above,

$$\langle E \rangle = \sum_i p_i E_i \tag{9.29}$$

is automatically satisfied by definition, so we have arrived at our maximum entropy probability distribution.

$$p_i = \frac{\mathrm{e}^{-\beta E_i}}{Z}, \tag{9.30}$$

with

$$Z = \sum_i \mathrm{e}^{-\beta E_i}. \tag{9.31}$$

Setting aside for a moment that we have not yet commented on what β is, the above probability distribution is the **Boltzmann distribution**. It is the maximal entropy probability distribution for a set of discrete states, each of which has an energy associated with it. To be able to use it to connect to probabilities of the two states of our protein, we need to figure out what β is.

9.3.1 Temperature as a derivative of entropy

A **system** (sometimes called a "body") is a collection of matter that can be in a set of microstates. A system has entropy S and an expectation for its energy, $\langle E \rangle$, often just called "energy" for short. The derivative of the entropy of a system with respect to its energy gives the reciprocal of a quantity called the **temperature**,

$$\frac{\mathrm{d}S}{\mathrm{d}\langle E \rangle} = \frac{1}{T}. \tag{9.32}$$

We have now formally defined temperature, and in so doing have established that it is a purely statistical quantity, in that it is only defined for a system, and *not* for a microstate of a system. We are now using the entropy as a *thermodynamic* quantity, not just informational, since we are considering the special case where we are applying entropy to a set of states that have energies associated with them.

With our definition of temperature in hand, we can approach the task of identifying β. Let us write down the entropy for the Boltzmann distribution as we have derived it so far.

$$\begin{aligned} S &= -K \sum_i p_i \ln p_i = -K \sum_i p_i \ln \frac{\mathrm{e}^{-\beta E_i}}{Z} = -K \sum_i p_i (-\beta E_i - \ln Z) \\ &= K\beta \sum_i p_i E_i + K \ln Z \sum_i p_i = K\beta \langle E \rangle + K \ln Z. \end{aligned} \tag{9.33}$$

Thus, we have

$$\frac{\partial S}{\partial \langle E \rangle} = K\beta = \frac{1}{T}. \tag{9.34}$$

So, for the Shannon entropy to be equal to the thermodynamic entropy, $K\beta = 1/T$. Thus, $\beta = 1/KT$. The constant K apparently imparts units to the temperature, since KT must have units of energy. In this context, we call the constant K the **Boltzmann constant**, and denote it as k_B or k. The Boltzmann constant has a value of

$$k_B = 1.38 \times 10^{-23}\,\mathrm{J/K}. \tag{9.35}$$

When we write the entropy as

$$S = -k_B \sum_i p_i \ln p_i, \tag{9.36}$$

it is referred to as the **Gibbs entropy**.

We will also use $\beta \equiv 1/k_B T$ in our calculations, since it turns out to be notationally convenient. Thus, we have

$$p_i = \frac{\mathrm{e}^{-E_i/k_B T}}{\sum_i \mathrm{e}^{-E_i/k_B T}}. \tag{9.37}$$

The quantity e^{-E_i/k_BT} represents an unnormalized probability and is referred to as a **Boltzmann weight**.

9.3.2 The Boltzmann distribution and our two-state protein

We have now worked out the probability of our protein being in the active versus inactive state. There are only two states, so the partition function is

$$Z = e^{-\beta E_{\text{active}}} + e^{-\beta E_{\text{inactive}}}. \tag{9.38}$$

Then, the probability that the protein is active is

$$p_{\text{active}} = \frac{e^{-\beta E_{\text{active}}}}{e^{-\beta E_{\text{active}}} + e^{-\beta E_{\text{inactive}}}} = \frac{1}{1 + e^{-\beta (E_{\text{inactive}} - E_{\text{active}})}}. \tag{9.39}$$

The probability of being active is dependent only on the *difference* in the energies of the active versus inactive states. In the limit of high temperature (small β), the protein has a 50/50 chance of being active of not. In the limit of low temperature (large β), $p_{\text{active}} = 1$ if $E_{\text{active}} < E_{\text{inactive}}$, and $p_{\text{active}} = 0$ if $E_{\text{active}} > E_{\text{inactive}}$.

10 From statistical to thermodynamical

In the previous lesson, we defined temperature to be a derivative of one statistical quantity, the entropy, with respect to another, the expectation of the energy, which we will refer to simply as the "energy." This is the first connection we drew between statistical quantities. In this lesson, we will derive more, and in the process expose **thermodynamic quantities**, properties are either the entropy itself, a differential thereof, or expectations of maximum-entropy distributions.

10.1 The extensivity of energy and entropy

You may recall in when we first introduced entropy that it is monotonically increasing in the number of outcomes of equal probability and further satisfies a composition law. The form of the entropy was derived from these desiderata, and we will use that form to investigate an important feature of the entropy, that it is first order homogeneous in its extensive variables. We will understand what that means in a moment.

Let us consider a thought experiment in which we have a system in which has microstates indexed by i. Then, the entropy of this system is

$$S_1 = -k_B \sum_i p_i \ln p_i. \tag{10.1}$$

Now, let's say we have another system that is the same size with the same available microstates, this time indexed by j. Of course, at any given time, these two systems might look different, with different microstates being populated, but they are identical in the sense that they have the same size, same number of particles, same microstates available, etc. The entropy of that system is

$$S_2 = -k_B \sum_j p_j \ln p_j. \tag{10.2}$$

Now, let's consider these two independent systems as the same system. The probability that the first system is in microstate i is p_i and the probability that the second system is in microstate j is p_j, and the probability that the composite system is in state (i,j) is $p_i p_j$. So, the entropy of the composite system is

$$\begin{aligned} S &= -k_B \sum_i \sum_j p_i p_j \ln p_i p_j = -k_B \sum_i \sum_j p_i p_j (\ln p_i + \ln p_j) \\ &= -k_B \sum_i \sum_j p_i p_j \ln p_i - k_B \sum_i \sum_j p_i p_j \ln p_j \end{aligned}$$

$$= -k_B \left[\sum_j p_j\right] \sum_i p_i \ln p_i - -k_B \left[\sum_i p_i\right] \sum_j p_j \ln p_j$$

$$= S_1 + S_2, \tag{10.3}$$

where we have used the fact that the bracketed terms are equal to one. So, we have shown that the entropy of a composite systems is additive over its constitutive subsystems. In this case, $S_1 = S_2$, so doubling the size of our system of interest also doubled the entropy.

I should clarify what I mean by the word "size" here. By "size," I mean all of the properties that doubled when we made the composite system out of two identical systems. For example, if the systems were particles in a box, I would have to double the number of particles and also double the volume of the box. Properties like the number of particles and the volume are **extensive properties**.

The energy (remember this is short for the *expectation* of the energy) has a similar relationship with the extensive properties of a system. Consider again our two systems comprising the composite system. If one system is in a microstate i with energy E_i and another is in a microstate j with energy E_j, then the total energy of the composite system is $E_i + E_j$. So, we can compute the expectation of the energy for the composite system as

$$\langle E \rangle = \sum_i \sum_j p_i p_j (E_i + E_j) = \sum_i \sum_j p_i p_j E_i + \sum_i \sum_j p_i p_j E_j$$

$$= \left[\sum_j p_j\right] \sum_i p_i E_i + \left[\sum_i p_i\right] \sum_j p_j E_j$$

$$= \langle E_1 \rangle + \langle E_2 \rangle. \tag{10.4}$$

As we have just shown, the entropy of a composite system is the sum of the entropy of its subsystems. In this sense, the entropy itself is also extensive. The same is true for the energy of a composite system; it is the sum of the energy of its subsystems. Energy is also extensive. Now, let N be the number of particles of a system, V be the volume of the system, X_1 be another extensive property of a system, X_2 be another extensive property, etc. The entropy may be a function of all of these, including the energy (which we already knew, since $\partial S / \partial \langle E \rangle) = 1/T$),

$$S = S(\langle E \rangle, V, N, X_1, X_2, \ldots). \tag{10.5}$$

If we were to increase all of the extensive parameters by a factor of a, we would have

$$S(a \langle E \rangle, a V, a N, a X_1, a X_2, \ldots) = a S(\langle E \rangle, V, N, X_1, X_2, \ldots). \tag{10.6}$$

Therefore, entropy is a first order homogeneous function of its extensive parameters. This is a very important result, which has the immediate consequence that Euler's theorem for homogeneous functions (recall Section 8.4) holds, such that

$$S(\langle E\rangle, V, N, X_1, X_2, \ldots) = \frac{\partial S}{\partial \langle E\rangle}\langle E\rangle + \frac{\partial S}{\partial V}V + \frac{\partial S}{\partial N}N + \frac{\partial S}{\partial X_1}X_1 + \frac{\partial S}{\partial X_2}X_2 + \ldots. \tag{10.7}$$

In computing the above partial derivatives, it is assumed that the other parameters are held constant. E.g., when computing $\partial S/\partial\langle E\rangle$, the volume, number of particles, X_1, X_2, etc., are all held constant. In most texts on thermodynamics, this is shown explicitly by surrounding the partial derivatives in parentheses and subscripting what is held constant, e.g.,

$$\frac{1}{T} = \left(\frac{\partial S}{\partial \langle E\rangle}\right)_{V,N,X_1,X_2,\ldots}. \tag{10.8}$$

We will sometimes use this notation when the clarity is needed, but will often simply write the partial derivatives.

10.2 Notation for the energy

Going forward, for notational and referential convenience, we will use the symbol E to mean the expectation of the energy $\langle E\rangle$. Any subscripted energy, e.g., E_i, denotes a microstate. We will refer to "energy" or "the energy" as the quantity with symbol E. In many texts, this energy is denoted as U and is called the **internal energy**, so named because it is the energy associated with the system of interest, in contrast to energy coming from outside of the system, such as gravitational potential energy. We will use the symbol E and refer to it simply as "the energy," as is more common in physics texts.

10.3 The total differentials of the energy and entropy

Based on our expression for the entropy and its extensive parameters above, (equation 10.7), we can write the the total differential of the entropy as

$$\mathrm{d}S = \frac{\partial S}{\partial E}\mathrm{d}E + \frac{\partial S}{\partial V}\mathrm{d}V + \frac{\partial S}{\partial N}\mathrm{d}N + \frac{\partial S}{\partial X_1}\mathrm{d}X_1 + \frac{\partial S}{\partial X_2}\mathrm{d}X_2 + \ldots. \tag{10.9}$$

We can rearrange this expression to instead write the total differential of the energy.

$$\mathrm{d}E = \frac{\partial E}{\partial S}\left(\mathrm{d}S - \frac{\partial S}{\partial V}\mathrm{d}V + \frac{\partial S}{\partial N}\mathrm{d}N + \frac{\partial S}{\partial X_1}\mathrm{d}X_1 + \frac{\partial S}{\partial X_2}\mathrm{d}X_2 + \ldots\right). \tag{10.10}$$

Clearly, because we did the simplest algebraic manipulation, these two expressions are the same. Thus, if we can write down a function of the energy as a function of the entropy and the other extensive parameters, we have exactly the same information as if we could write the entropy as a function of the energy and its other extensive parameters.

10.4 Intensive parameters

In contrast to extensive parameters, **intensive properties** are unchanged when we change the size of a system. Temperature and pressure are examples. Because they are unchanged with changes in extensive parameters, they are zero order homogeneous functions of the extensive parameters of a system. As an example, consider the temperature, which we may write as a function of the system's extensive properties as $T(S, V, N, X_1, X_2, \ldots)$. Then,

$$T(a\,S, a\,V, a\,N, a\,X_1, a\,X_2, \ldots) = T(S, V, N, X_1, X_2, \ldots). \tag{10.11}$$

It can be shown (and you will in your homework) that if X and Y are extensive parameters, then X/Y and $\partial X/\partial Y$ are intensive. Referring to the expression for the entropy above,

$$S(E, V, N, X_1, X_2, \ldots) = \frac{\partial S}{\partial E} E + \frac{\partial S}{\partial V} V + \frac{\partial S}{\partial N} N + \frac{\partial S}{\partial X_1} X_1 + \frac{\partial S}{\partial X_2} X_2 + \ldots, \tag{10.12}$$

we see that the entropy may be written as the sum of product-pairs, each one the product of an intensive property and an extensive one. These intensive-extensive pairs are called **conjugate thermodynamic variables**.

$$\frac{\partial S}{\partial E}, \frac{\partial S}{\partial V}, \frac{\partial S}{\partial N}, \frac{\partial S}{\partial X_1}, \frac{\partial S}{\partial X_2}, \ldots$$

are all intensive properties. We can *define* them to have names and associated symbols. They are, starting with the one we have already seen,

$$\frac{\partial S}{\partial E} = \frac{1}{T}, \tag{10.13}$$

$$\frac{\partial S}{\partial V} = \frac{p}{T}, \tag{10.14}$$

$$\frac{\partial S}{\partial N} = -\frac{\mu}{T}, \tag{10.15}$$

$$\frac{\partial S}{\partial X_i} = -\frac{\xi_i}{T}, \tag{10.16}$$

where p is referred to as the thermodynamic **pressure**, μ is the **chemical potential**, and ξ_i denotes a generic intensive property. With the exception of temperature, intensive properties are denoted with lower case symbols and extensive properties with upper case symbols.

With these names in hand, we can write the total differentials for the entropy and the energy as

$$\mathrm{d}S = \frac{1}{T}\,\mathrm{d}E + \frac{p}{T}\,\mathrm{d}V - \frac{\mu}{T}\,\mathrm{d}N - \frac{\xi_1}{T}\,\mathrm{d}X_1 - \frac{\xi_2}{T}\,\mathrm{d}X_2 - \ldots, \tag{10.17}$$

$$\mathrm{d}E = T\,\mathrm{d}S - p\,\mathrm{d}V + \mu\,\mathrm{d}N + \xi_1\,\mathrm{d}X_1 + \xi_2\,\mathrm{d}X_2 + \ldots. \tag{10.18}$$

We can also use Euler's theorem for first order homogeneous functions to write the energy as

$$E = TS - pV + \mu N + \xi_1 X_1 + \xi_2 X_2 + \ldots. \tag{10.19}$$

Thus, we see that temperature is conjugate to entropy, negative pressure to volume, chemical potential to number of particles, and so on.

10.5 Thermodynamic quantities

It is important to recall, again, that all of the properties in the above total differentials are *statistical* quantities. That is, they are either the entropy itself, a differential thereof, or expectations. They are all *properties*, or summaries, of the probability distribution of microstates of a system. Such quantities are called **thermodynamic quantities**, and the study of thermodynamics is the study of these summaries of the underlying probability distribution of the microstates of a system.

(10.20)

11 Thermodynamic potentials

In this lesson, we will derive will explore **thermodynamic potentials**. These are Legendre transforms of the energy, as we will expose momentarily.

11.1 The free energy

As we start down the road to introduce thermodynamic potentials, recall that we derived the Boltzmann distribution,

$$P_i = \frac{\mathrm{e}^{-\beta E_i}}{Z}, \quad Z = \sum_i \mathrm{e}^{-\beta E_i} \tag{11.1}$$

by maximizing entropy subject to the normalization constraint and by considering that each microstate i has an energy associated with it and an expectation for the energy exists. We derived a relationship between the entropy and the energy,

$$S = k_B\, \beta \langle E \rangle + k_B\, \ln Z. \tag{11.2}$$

The dimensionless quantity $\ln Z$, the natural logarithm of the partition function, appears in this expression. It turns out that this is a very important quantity, and its importance will become clear as we explore its relationship to other statistical quantities. At the risk of putting the cart before the horse because I know where this is all headed, I am going to define a quantity called the **free energy**, often referred to (particularly by chemists) as the **Helmholtz free energy**,[13]

$$F = -k_B T \ln Z = -\frac{\ln Z}{\beta}. \tag{11.3}$$

We will discuss why this is called a free energy soon, but for now, we can think of this as an energy proportional to the logarithm of the partition function, $\ln Z$.

To understand what this free energy is, we note two properties derivable from the Boltzmann distribution.

$$F = E - TS, \tag{11.4}$$

$$S = -\frac{\partial F}{\partial T}. \tag{11.5}$$

[13] Chemists often use the symbol A for the free energy instead of F, after the German word Arbeit, which means "work." The connection to work will be made clear soon as well.

You can derive these yourself (now, or in the homework!). The first equation suggests that F is a Legendre transform of the energy. This can be seen by recalling from our definition of Legendre transforms in Section 8.3,

$$\text{Legendre-transformed E} = E - \frac{\partial E}{\partial S} S = E - TS, \tag{11.6}$$

since the temperature is defined as

$$T = \frac{\partial E}{\partial S}. \tag{11.7}$$

Indeed, F is behaving like a Legendre-transformed energy. Then, we have

$$\mathrm{d}F = \mathrm{d}E - T\mathrm{d}S - S\mathrm{d}T. \tag{11.8}$$

Since

$$\mathrm{d}E = T\mathrm{d}S - p\mathrm{d}V + \mu\,\mathrm{d}N + \xi_1 \mathrm{d}X_1 + \xi_2 \mathrm{d}X_2 + \ldots, \tag{11.9}$$

we have

$$\mathrm{d}F = -S\mathrm{d}T - p\mathrm{d}V + \mu\,\mathrm{d}N + \xi_1 \mathrm{d}X_1 + \xi_2 \mathrm{d}X_2 + \ldots. \tag{11.10}$$

Thus, to verify that F is a Legendre transform of the energy, we must have that

$$\left(\frac{\partial F}{\partial T}\right)_{V,N,X_i} = -S, \tag{11.11}$$

which you can also show. Therefore, F is indeed a Legendre transform of the energy. As a Legendre transform, it has the same information as the energy E, which means it has the same information as the entropy S.

Recalling relations of first order homogeneous functions, we identify that the free energy is a function of T, V, N, X_1, X_2, ...;

$$F = F(T, V, N, X_1, X_2, \ldots). \tag{11.12}$$

For notational convenience going forward, we will focus mainly on the variables T, S, p, V, μ, and N, and will just keep in mind that we can have arbitrary other parameters ξ_i, X_i, so that we write

$$F = F(T, V, N), \tag{11.13}$$

with the other parameters implied where applicable. Thus, we say that the free energy is a function of temperature, volume, and number of particles.

11.2 The Gibbs free energy

Let us now consider the case where each microstate has associated with it a volume; that is the volume may also vary from microstate to microstate in addition to the energy. Then, we enforce that there exists an expectation of the volume,

$$\langle V \rangle = \sum_i V_i p_i. \tag{11.14}$$

In this case, our Lagrangian is

$$\begin{aligned}\mathcal{L} &= S - \alpha \left(1 - \sum_i p_i\right) - \beta \left(\langle E \rangle - \sum_i E_i p_i\right) - \gamma \left(\langle V \rangle - \sum_i V_i p_i\right) \\ &= -k_B \sum_i p_i \ln p_i - \alpha \left(1 - \sum_i p_i\right) - \beta \left(\langle E \rangle - \sum_i E_i p_i\right) - \gamma \left(\langle V \rangle - \sum_i V_i p_i\right),\end{aligned} \tag{11.15}$$

where we have introduced another Lagrange multiplier γ. Evaluating

$$\frac{\partial \mathcal{L}}{\partial p_j} = 0 \ \ \forall j \tag{11.16}$$

and rearranging yields

$$p_i = \mathrm{e}^{-1-\alpha/k_B} \mathrm{e}^{-\beta E_i/k_B} \mathrm{e}^{-\gamma V_i/k_B}. \tag{11.17}$$

Reassigning the constants such at $\alpha \leftarrow -1 - \alpha/k_B$, $\beta \leftarrow \beta/k_B$, and $\beta\gamma \leftarrow \gamma/k_B$ yields

$$p_i = \mathrm{e}^{\alpha} \mathrm{e}^{-\beta E_i} \mathrm{e}^{-\beta\gamma V_i}. \tag{11.18}$$

We chose to redefine γ such that each term in the exponents appear as β times an energy. Enforcing the normalization condition forces the value of α to be such that

$$p_i = \frac{\mathrm{e}^{-\beta(E_i + \gamma V_i)}}{\mathcal{Z}}, \tag{11.19}$$

where

$$\mathcal{Z} = \sum_i \mathrm{e}^{-\beta(E_i + \gamma V_i)}. \tag{11.20}$$

Akin to the free energy, we posit that we can define the **Gibbs free energy** to be

$$G = -k_B T \ln \mathcal{Z}. \tag{11.21}$$

Recall that the pressure is

$$p = T\frac{\partial S}{\partial \langle V \rangle}, \tag{11.22}$$

where we have explicitly written the thermodynamic quantity of volume V as an expectation over the probability distribution we have defined above. To connect the pressure to the Lagrange multiplier γ, we need to write the entropy as a function of $\langle V \rangle$ so we can compute the derivative. We proceed as we did for Equation (9.33) when we derived an expression for the entropy as a function of energy.

$$\begin{aligned} S &= -k_B \sum_i p_i \ln p_i = -k_B \sum_i p_i \ln \frac{\mathrm{e}^{-\beta(E_i+\gamma V_i)}}{\mathcal{Z}} \\ &= -k_B \sum_i p_i(-\beta(E_i+\gamma V_i) - \ln \mathcal{Z}) \\ &= k_B\beta \left(\sum_i E_i p_i + \gamma \sum_i V_i p_i \right) + k_B \ln \mathcal{Z} \sum_i p_i \\ &= k_B\beta \langle E \rangle + k_B \beta \gamma \langle V \rangle + k_B \ln \mathcal{Z}. \end{aligned} \tag{11.23}$$

Using this result, we can compute

$$T\frac{\partial S}{\partial \langle V \rangle} = k_B T \beta \gamma = \gamma. \tag{11.24}$$

Thus, we have that the Lagrange multiplier γ is the pressure (which is why we chose the sign we did when defining it), such that

$$p_i = \frac{\mathrm{e}^{-\beta(E_i+pV_i)}}{\mathcal{Z}}, \tag{11.25}$$

with

$$\mathcal{Z} = \sum_i \mathrm{e}^{-\beta(E_i+pV_i)}, \tag{11.26}$$

which also gives that

$$S = k_B\,\beta(\langle E \rangle + p\langle V \rangle) + k_B \ln \mathcal{Z}. \tag{11.27}$$

Multiplying both sides by the temperature T, we get

$$TS = \langle E \rangle + p\langle V \rangle - G, \tag{11.28}$$

or, rearranging and dropping the expectation brackets,

$$G = E - TS + pV = F + pV. \tag{11.29}$$

Thus, the Gibbs free energy is a Legendre transform of the free energy.

The total differential of the Gibbs free energy is then

$$\begin{aligned} \mathrm{d}G &= \mathrm{d}F + p\mathrm{d}V + V\mathrm{d}p \\ &= -S\mathrm{d}T + V\mathrm{d}p + \mu\,\mathrm{d}N + \xi_1\mathrm{d}X_1 + \xi_2\mathrm{d}X_2 + \ldots. \end{aligned} \tag{11.30}$$

The variables of the Gibbs free energy are $G = G(T, p, N)$. As a Legendre transform of the free energy, which itself has all of the thermodynamic information of a system, so too does the Gibbs free energy contain all of the thermodynamic information.

11.3 Whence "free energy?"

Why are the free energy and Gibbs free energy called "free energies?" To answer this question, we need to step back for a moment and think about the energy of a system. The energy of a system changes by putting energy in or taking energy out (just like in a material balance, energy is conserved, so we have to think about what is put in and taken out). We can think of two ways in which we can put energy in to a system. One way is to mechanically manipulate the system, e.g., by compressing it or moving it in a gravitational field. This mechanical manipulation is called **work**, which we will denote as W. Another way is to move energy from a neighboring system at a higher temperature than the system of interest. We have already seen this kind of energy transfer; it served to bring the temperatures of the two systems to the same value. Transfer of energy in this manner is called **heat**, which we will denote as Q. So, we can write the total differential of energy as

$$\mathrm{d}E = đQ + đW. \tag{11.31}$$

I was careful here to write a slash through the d's because these are strictly not total differentials; only the energy has a total differential. We cannot talk about the heat of a system or the work of a system, but we can talk about the energy of a system. If an system has some energy E_1 and then conditions are changed such that it has energy E_2, then the total amount of heat *plus* the total amount of work is $E_2 - E_1$. That is to say, we can only talk about heat and work as it pertains to *changes* in the energy of a system.

We have also that, provided we are operating under conditions where thermodynamics apply; that is that the state of the system is such that the entropy is well-defined, said to be in **equilibrium**,

$$\mathrm{d}E = T\,\mathrm{d}S - p\,\mathrm{d}V + \mu\,\mathrm{d}N + \xi_1\,\mathrm{d}X_1 + \xi_2\,\mathrm{d}X_2 + \ldots. \tag{11.32}$$

We can imagine that changing the volume of a system is a way to add energy by doing mechanical work. The system may also do chemical work, e.g., by changing N, or

other kinds of work by changing X_1, X_2, etc. Then, for a change in these properties over time,

$$\frac{\mathrm{d}W}{\mathrm{d}t} = -p\frac{\mathrm{d}V}{\mathrm{d}t} + \mu\frac{\mathrm{d}N}{\mathrm{d}t} + \xi_1\frac{\mathrm{d}X_1}{\mathrm{d}t} + \xi_2\frac{\mathrm{d}X_2}{\mathrm{d}t} + \ldots. \tag{11.33}$$

Now, if we do this work in such a way that we maintain a thermal equilibrium, then

$$\frac{\mathrm{d}E}{\mathrm{d}t} = T\frac{\mathrm{d}S}{\mathrm{d}t} - p\frac{\mathrm{d}V}{\mathrm{d}t} + \mu\frac{\mathrm{d}N}{\mathrm{d}t} + \xi_1\frac{\mathrm{d}X_1}{\mathrm{d}t} + \xi_2\frac{\mathrm{d}X_2}{\mathrm{d}t} + \ldots = \frac{\mathrm{d}W}{\mathrm{d}t} + \frac{\mathrm{d}Q}{\mathrm{d}t}. \tag{11.34}$$

Comparing with our expression for $\mathrm{d}W/\mathrm{d}t$, we see that, under these conditions,

$$\frac{\mathrm{d}Q}{\mathrm{d}t} = T\frac{\mathrm{d}S}{\mathrm{d}t}. \tag{11.35}$$

Note that this assumes we operate such that thermal equilibrium is maintained, but if we do not quite manage that, the entropy must increase to to processes other than heat transfer, so

$$\frac{\mathrm{d}Q}{\mathrm{d}t} \leq T\frac{\mathrm{d}S}{\mathrm{d}t}. \tag{11.36}$$

In this class, we will usually consider processes for which we maintain thermal equilibrium, and the above holds with equality.

Now, assuming we operate such that entropy gain is due entirely to heat, we have

$$\mathrm{d}W = \mathrm{d}E - \mathrm{d}Q = \mathrm{d}E - T\mathrm{d}S. \tag{11.37}$$

If we do the work **isothermally**, that is while holding temperature constant, we have

$$T\mathrm{d}S = \mathrm{d}(TS) = \cancelto{0}{S\mathrm{d}T} + T\mathrm{d}S. \tag{11.38}$$

Thus,

$$\mathrm{d}W = \mathrm{d}(E - TS) = \mathrm{d}F, \tag{11.39}$$

since the free energy F is $F = E - TS$. So, the work done in an isothermal process where equilibrium is maintained is equal to the free energy. That is, the free energy is the amount of energy that is available, or "free," to do work.

It's an archaic name, relating back to the early days of thermodynamics when the work involved expansion and compression of gasses to power steam engine, but understanding where the name comes from allowed us to think about the concepts of work and heat. Those concepts allow us to proceed to another important concept.

11.4 Equilibrium as a minimization of free energy

We have seen that the quantities E, V, N, etc., at equilibrium are those that maximize the entropy. We now show that equilibrium is equivalently achieved, under certain conditions, when the free energy is minimal.

We also just showed that

$$\frac{\mathrm{d}Q}{\mathrm{d}t} \leq T\frac{\mathrm{d}S}{\mathrm{d}t}, \tag{11.40}$$

with equality in equilibrium conditions. We also have, from Equations (11.33) and (11.34) that

$$\frac{\mathrm{d}Q}{\mathrm{d}t} = \frac{\mathrm{d}E}{\mathrm{d}t} + p\frac{\mathrm{d}V}{\mathrm{d}t} - \mu\frac{\mathrm{d}N}{\mathrm{d}t} - \xi_1\frac{\mathrm{d}X_1}{\mathrm{d}t} - \xi_2\frac{\mathrm{d}X_2}{\mathrm{d}t} - \dots. \tag{11.41}$$

Thus, we have

$$\frac{\mathrm{d}E}{\mathrm{d}t} + p\frac{\mathrm{d}V}{\mathrm{d}t} - \mu\frac{\mathrm{d}N}{\mathrm{d}t} - \xi_1\frac{\mathrm{d}X_1}{\mathrm{d}t} - \xi_2\frac{\mathrm{d}X_2}{\mathrm{d}t} - \ldots \leq T\frac{\mathrm{d}S}{\mathrm{d}t}. \tag{11.42}$$

If the changes of the system happen under conditions where the temperature, volume, number of particles, etc., are held constant, then we may write

$$\frac{\mathrm{d}(E-TS)}{\mathrm{d}t} = \frac{\mathrm{d}F}{\mathrm{d}t} \leq 0, \tag{11.43}$$

again, with equality holding at equilibrium. Therefore, equilibrium is achieved when the free energy is *minimal* for constant temperature and constant volume processes. Similarly, we can derive that for processes with constant temperature and constant pressure, the Gibbs free energy is minimized.

11.5 Generalized potentials

Quantities that are Legendre transforms of the energy are called **thermodynamic potentials**. They contain all of the the thermodynamic information of a system. We have so far seen the free energy and the Gibbs free energy as thermodynamic potentials. Let us think for a minute about how they were derived. We started by considering what extensive parameters may vary among the microstates of a system. We then imposed that an expectation of the varying quantities must exist via a Lagrange multiplier in an entropy maximization problem. For the free energy, we allowed the energy to vary from microstate to microstate, and for the Gibbs free energy, we allowed the energy and volume to vary. Upon maximizing the entropy, we find an equilibrium probability distribution of microstates. We then used this probability mass function to compute the appropriate expectations, allowing us to differentiate

the entropy with respect to expectations, thereby connecting the Lagrange multipliers to thermodynamic quantities given by derivatives of the entropy. Finally, we could identify that the quantity given by the negative log of the partition function, multiplied by the thermal energy $k_B T$, is a Legendre transform of the energy.

A more general result follows. Let us say that in addition to the energy, quantities X_1, X_2, etc., may vary from microstate to microstate. For notational convenience, we will consider only one such quantity, X, noting that the analysis trivially generalizes to more (or even less, which is what we did for the free energy). The quantity X can be any extensive variable. The Lagrangian for the entropy maximization problem is

$$\begin{aligned}\mathcal{L} = -k_B \sum_i p_i \ln p_i - \alpha \left(1 - \sum_i p_i\right) - \beta \left(\langle E \rangle - \sum_i E_i p_i\right) \\ - \beta \xi \left(\langle X \rangle - \sum_i X_i p_i\right),\end{aligned} \tag{11.44}$$

where $\beta \xi$ is the Lagrange multiplier for the quantity X. Solving the optimization problem yields

$$p_i = \frac{\mathrm{e}^{-\beta Ei}\, \mathrm{e}^{-\beta \xi X_i}}{\mathcal{Z}}, \tag{11.45}$$

where the partition function $\mathcal{Z}$ is given by

$$\mathcal{Z} = \sum_i \mathrm{e}^{-\beta E_i}\, \mathrm{e}^{-\beta \xi X_i}. \tag{11.46}$$

Using this probability mass function, the entropy can be written as

$$\begin{aligned}S &= k_B \beta \langle E \rangle + k_B \beta \xi \langle X \rangle + k_B \ln \mathcal{Z} \\ &= \frac{1}{T} \langle E \rangle + \frac{\xi}{T} \langle X \rangle + k_B \ln \mathcal{Z}.\end{aligned} \tag{11.47}$$

It is clear, then, that the Lagrange multipliers are intensive properties given by derivative of the entropy,

$$\begin{aligned}\frac{1}{T} &= \frac{\partial S}{\partial \langle E \rangle}, \\ \frac{\xi}{T} &= \frac{\partial S}{\partial \langle X \rangle}.\end{aligned} \tag{11.48}$$

If we define our potential to be $\mathcal{F} = -k_B T \ln \mathcal{Z}$, such that

$$S = \frac{1}{T} \langle E \rangle + \frac{\xi}{T} \langle X \rangle - \frac{1}{T} \mathcal{F}. \tag{11.49}$$

Recalling again that the entropy is a first order homogeneous function of the extensive parameters $\langle E \rangle$ and $\langle X \rangle$, $\mathcal{F}$ is also extensive. Rearranging, we have

$$\mathcal{F} = \langle E \rangle - TS + \xi \langle X \rangle. \tag{11.50}$$

We identify the result as a Legendre transform of the energy, such that

$$\mathrm{d}\mathcal{F} = \mathrm{d}\mathcal{F}(T, \xi) = -S\,\mathrm{d}T + x\mathrm{d}\xi, \tag{11.51}$$

where $X = \langle X \rangle$.

11.6 The enthalpy

Let us consider now another Legendre transform of the energy that we occasionally use in assays in biochemistry. We will use the $(-p, V)$ conjugate pair and define the **enthalpy** H as

$$H = E + pV. \tag{11.52}$$

Computing the total differential, the enthalpy is

$$\begin{aligned} \mathrm{d}H &= \mathrm{d}E + p\mathrm{d}V + V\mathrm{d}p \\ &= T\mathrm{d}S - p\mathrm{d}V + \mu\,\mathrm{d}N + p\mathrm{d}V + V\mathrm{d}p \\ &= T\mathrm{d}S + V\mathrm{d}p + \mu\,\mathrm{d}N. \end{aligned} \tag{11.53}$$

Just as a reminder, we could keep other parameters as well.

$$\mathrm{d}H = T\mathrm{d}S + V\mathrm{d}p + \mu\,\mathrm{d}N + \xi_1\mathrm{d}X_1 + \xi_2\mathrm{d}X_2 + \ldots, \tag{11.54}$$

but for notational convenience, we usually write $H = H(S, p, N)$.

11.7 The Gibbs-Duhem relation

Let us now consider a *total* Legendre transform of the energy. That is, we will perform a Legendre transform using all of the conjugate pairs. To do this, we will first write the total differential of the energy as

$$\mathrm{d}E = T\mathrm{d}S - p\mathrm{d}V + \mu\,\mathrm{d}N + \xi_1\,\mathrm{d}X_1 + \xi_2\,\mathrm{d}X_2 + \cdots. \tag{11.55}$$

Next, we note from Euler's theorem that

$$E = TS - pV + \mu N + \xi_1 X_1 + \xi_2 X_2 + \cdots, \tag{11.56}$$

from which we can compute the total differential as

$$\begin{aligned} \mathrm{d}E = \underline{T\mathrm{d}S} - S\mathrm{d}T - \underline{p\mathrm{d}V} - V\mathrm{d}p + \underline{\mu\,\mathrm{d}N} + N\mathrm{d}\mu \\ + \underline{\xi_1\,\mathrm{d}X_1} + X_1\,\mathrm{d}\xi_1 + \underline{\xi_2\,\mathrm{d}X_2} + X_2\,\mathrm{d}\xi_2 + \cdots . \end{aligned} \tag{11.57}$$

Equating the two expressions for the total differential of the energy, $\mathrm{d}E$, and noting that the underlined terms appear in both, we have

$$-S\mathrm{d}T - V\mathrm{d}p + N\mathrm{d}\mu + X_1\,\mathrm{d}\xi_1 + X_2\,\mathrm{d}\xi_2 + \cdots = 0, \tag{11.58}$$

a result known as the **Gibbs-Duhem relation**. It says that the total differential of the total Legendre transform of the energy is zero.

12 Thermodynamic relations

Now that we have laid the groundwork for the study of thermodynamics, introducing the key concepts of entropy, energy, and thermodynamic potentials, as well as work and heat, we can see how these quantities are related.

We already know that the thermodynamic potentials are Legendre transforms of the energy. For example,

$$F = E - TS, \tag{12.1}$$

$$H = E + pV, \tag{12.2}$$

$$G = F + pV = H - TS = E - TS + pV. \tag{12.3}$$

We know that the temperature is given by the derivative of the entropy with respect to the energy.

$$\frac{\partial S}{\partial E} = \frac{1}{T}. \tag{12.4}$$

We also know that the intensive parameters are derivatives of the entropy with respect to their extensive conjugate (modulo temperature) with energy held constant. They are similarly defined as derivatives of the energy with entropy held constant.

$$p = T\left(\frac{\partial S}{\partial V}\right)_{E,N,\ldots} = \left(\frac{\partial E}{\partial V}\right)_{S,N,\ldots}, \tag{12.5}$$

$$\mu = T\left(\frac{\partial S}{\partial N}\right)_{E,V,\ldots} = \left(\frac{\partial E}{\partial N}\right)_{S,V,\ldots}, \tag{12.6}$$

$$\xi = T\left(\frac{\partial S}{\partial X}\right)_{E,V,N,\ldots} = \left(\frac{\partial E}{\partial X}\right)_{S,V,N,\ldots}. \tag{12.7}$$

Finally, we know that if we operate such that thermal equilibrium is maintained while changing conditions, then the heat and entropy differentials are related by

$$\mathrm{d}Q = T\mathrm{d}S. \tag{12.8}$$

Furthermore, if we change conditions such that temperature is held constant, the work is given by

$$\mathrm{d}W = \mathrm{d}F. \tag{12.9}$$

Generally,

$$\mathrm{d}E = đQ + đW, \tag{12.10}$$

with the path-dependent đ's changing to total differentials if we operate maintaining thermal equilibrium.

With these relations in hand, we proceed to explore more.

12.1 Heat capacity

Let's say we wish to see how much heat is required to raise the temperature of a system by one temperature unit. To answer this, from the relationship between heat and entropy, we can write

$$\frac{\partial Q}{\partial T} = T\frac{\partial S}{\partial T}, \tag{12.11}$$

which holds for processes where thermal equilibrium is maintained throughout. We define the **heat capacity** C as

$$C = T\frac{\partial S}{\partial T}. \tag{12.12}$$

Then, the heat necessary to change the temperature from T_1 to T_2 is

$$Q = \int_{T_1}^{T_2} \mathrm{d}T\, C. \tag{12.13}$$

Of course, the amount of heat to raise the temperature will depend on how the procedure is done (beyond just ensuring that it is in thermal equilibrium as the process proceeds), and we should be careful how we define our derivative of the entropy. For example, if the process is done with the volume of the system held constant, then

$$C \equiv C_v = T\left(\frac{\partial S}{\partial T}\right)_V. \tag{12.14}$$

Similarly, if the process is done with the pressure of the system held constant, then

$$C \equiv C_p = T\left(\frac{\partial S}{\partial T}\right)_p. \tag{12.15}$$

Recalling the total differential of the energy,

$$\mathrm{d}E = T\mathrm{d}S - p\mathrm{d}V + \mu\,\mathrm{d}N, \tag{12.16}$$

it is apparent that

$$\left(\frac{\partial E}{\partial T}\right)_{V,N} = T\left(\frac{\partial S}{\partial T}\right)_{V,N} = C_v. \tag{12.17}$$

Therefore, at constant volume, the incremental gain in energy is given by an equivalent quantity of heat. This makes sense, because holding the volume constant constrains the system to be absent of mechanical work (assuming there are no other mechanical variables), so all of the energy change is present as heat.

Similarly, recalling the total differential of the enthalpy,

$$\mathrm{d}H = T\mathrm{d}S + V\mathrm{d}p + \mu\,\mathrm{d}N, \tag{12.18}$$

it is apparent that

$$\left(\frac{\partial H}{\partial T}\right)_{p,N} = T\left(\frac{\partial S}{\partial T}\right)_{p,N} = C_p. \tag{12.19}$$

Because the volume may change, some energy is used to do work, so not all of heat is used to change the energy. The enthalpy is $H = E + pV$, which is precisely the energy minus p-V work.

12.1.1 The positivity of the heat capacity

It matches our lived experience that the heat capacity is positive, since that implies that adding heat to a system raises its temperature. But must this be true? And if so, why?

To investigate, we recall that thermodynamic equilibrium occurs when the entropy is maximal. The entropy is given by the Gibbs entropy, determined by the probability mass function of microstates of a system. We can also write the entropy as a function of expectations of this probability distribution, which comprise the thermodynamic parameters. So, just as the entropy is a concave function of the probabilities, it must be a concave function of its thermodynamic parameters.

Since the entropy is a function of the energy, volume, number of particles, and other extensive parameters, we write it as $S = S(E, V, N, \ldots)$. Then, in order to be a concave function of these parameters, we must have the matrix

$$\nabla\nabla S = \begin{pmatrix} \left(\frac{\partial^2 S}{\partial E^2}\right)_{V,N} & \left(\frac{\partial^2 S}{\partial E\,\partial V}\right)_{N} & \left(\frac{\partial^2 S}{\partial E\,\partial N}\right)_{E} \\ \left(\frac{\partial^2 S}{\partial E\,\partial V}\right)_{N} & \left(\frac{\partial^2 S}{\partial V^2}\right)_{E,N} & \left(\frac{\partial^2 S}{\partial V\,\partial N}\right)_{E} \\ \left(\frac{\partial^2 S}{\partial E\,\partial N}\right)_{V} & \left(\frac{\partial^2 S}{\partial V\,\partial N}\right)_{E} & \left(\frac{\partial^2 S}{\partial N^2}\right)_{E,V} \end{pmatrix} \tag{12.20}$$

be negative definite. A necessary condition is that each of the entries in the diagonal are negative. Consider the upper left entry.

$$\left(\frac{\partial^2 S}{\partial E^2}\right)_{V,N} = \left(\frac{\partial}{\partial E}\,\frac{1}{T}\right)_{V,N} = -\frac{1}{T^2}\left(\frac{\partial T}{\partial E}\right)_{V,N} = -\frac{1}{T^2\,C_v} < 0, \tag{12.21}$$

where we have used the facts that $\partial S/\partial E = 1/T$ and $(\partial E/\partial T)_V = C_v$. Thus, in order for the entropy to be concave and therefore maximal, we must have $C_v > 0$.

Systems were the constant-volume heat capacity becomes negative are said to be **unstable**. More generally, an unstable system is one for which the entropy ceases to be a concave function of one or more of its extensive thermodynamic variables.

12.1.2 Stability conditions and positivity of C_p

What about C_p? We can take a similar approach, but it is more convenient to do so using a Legendre transform of the entropy. The entropy is a concave function of its variables, all of which are extensive. Consider now the Legendre transform of the entropy. Traditionally, the symbol S is overloaded for Legendre transforms of entropy, with $S = S(E, V, N)$ being the entropy and $S[1/T] = S[1/T](T, V, N)$ being the Legendre transform. The Legendre transform is

$$S[1/T] = S - \frac{E}{T}, \tag{12.22}$$

and its differential is

$$\mathrm{d}S[1/T] = -E\,\mathrm{d}\left(\frac{1}{T}\right) + p\,\mathrm{d}V + \mu\,\mathrm{d}N. \tag{12.23}$$

Now, consider an arbitrary Legendre transform of the entropy,

$$S[\xi/T] = S - \frac{\xi}{T}X, \tag{12.24}$$

We know that the entropy must be a concave function of X such that

$$\frac{\partial^2 S}{\partial X^2} = \frac{\partial(\xi/T)}{\partial X} < 0. \tag{12.25}$$

Now consider the second derivative of $S[\xi/T]$ with respect to ξ/T. Since

$$\mathrm{d}S[\xi/T] = -X\,\mathrm{d}\left(\frac{\xi}{T}\right) + \cdots, \tag{12.26}$$

$$\frac{\partial^2 S[\xi/T]}{\partial(\xi/T)^2} = -\frac{\partial X}{\partial(\xi/T)} = -\frac{1}{\dfrac{\partial(\xi/T)}{\partial X}}. \tag{12.27}$$

Since we have already established that the denominator must be negative for a stable system, the second derivative of the Legendre transformed entropy must be positive. Generally, a Legendre transformed entropy must be a concave function of its extensive parameters and a convex function of its intensive parameters. This follows directly from the nature of Legendre transforms.

It is further true (here stated without proof) that stability requires that the energy is a convex function of its variables (all of which are extensive) and that Legendre transforms of the energy are convex functions of their extensive variables and concave functions of their intensive variables.

Using this latter property, we address the heat capacity for constant-pressure processes. Recalling that

$$\mathrm{d}G = -S\mathrm{d}T + V\mathrm{d}p + \mu\,\mathrm{d}N, \tag{12.28}$$

we can consider

$$\left(\frac{\partial^2 G}{\partial T^2}\right)_{p,N} = -\left(\frac{\partial S}{\partial T}\right)_{p,N} = -\frac{1}{T}C_p < 0. \tag{12.29}$$

The last inequality holds because the Gibbs free energy must be a concave function of its intensive parameters, including the temperature T. Thus, we also have $C_p > 0$.

12.2 Susceptibilities

The heat capacities C_v and C_p are useful because they can be measured. In fact, we will soon use them to interpret experiments on protein denaturation. However, heat capacities are not the only measurable derivatives of the entropy or energy. In general, a **susceptibility** is a quantity related to the derivative of an extensive property with respect to an intensive property. We have seen two already, $C_v = (\partial E/\partial T)_V$ and $C_p = (\partial E/\partial T)_p$.

Let us now investigate two more related to mechanical changes, specifically changes of volume, of a system. The **isothermal compressibility** κ_T gives the fractional change in the volume of a system upon a change in pressure under isothermal conditions, and is given by

$$\kappa_T = -\frac{1}{V}\left(\frac{\partial V}{\partial p}\right)_T. \tag{12.30}$$

The isothermal compressibility is positive for stable systems. The **coefficient of thermal expansion** α is the fractional change in volume upon raising the temperature while pressure is held constant. It is given by

$$\alpha = \frac{1}{V}\left(\frac{\partial V}{\partial T}\right)_p. \tag{12.31}$$

12.3 Relating the susceptibilities

We have seen definitions of C_p, C_v, κ_T, and α, which makes it clear how they are related to thermodynamic variables and their derivatives, but how are they related to

each other? It turns out that addressing this question can be conveniently done with some useful mathematical tools that we now introduce.

12.3.1 The triple product rule

The **triple product rule**, which we will not derive here, follows from the implicit function theorem and says that if three variables can be related by a function $f(x, y, z) = 0$, which is very often the case in thermodynamics, then

$$\left(\frac{\partial x}{\partial y}\right)_z \left(\frac{\partial y}{\partial z}\right)_x \left(\frac{\partial z}{\partial x}\right)_y = -1. \tag{12.32}$$

As an example, we can relate the coefficient of thermal expansion to other thermodynamic derivatives using this formula.

$$\left(\frac{\partial V}{\partial T}\right)_p \left(\frac{\partial T}{\partial p}\right)_V \left(\frac{\partial p}{\partial V}\right)_T = -1, \tag{12.33}$$

which leads to

$$\alpha = \frac{1}{V}\left(\frac{\partial V}{\partial T}\right)_p = -\frac{1}{V}\left[\left(\frac{\partial T}{\partial p}\right)_V \left(\frac{\partial p}{\partial V}\right)_T\right]^{-1} = -\frac{1}{V}\left(\frac{\partial p}{\partial T}\right)_V \left(\frac{\partial V}{\partial p}\right)_T. \tag{12.34}$$

12.3.2 Jacobians

A 2×2 **Jacobian** is defined as

$$\frac{\partial(u, v)}{\partial(x, y)} \equiv \det \begin{pmatrix} \left(\frac{\partial u}{\partial x}\right)_y & \left(\frac{\partial u}{\partial y}\right)_x \\ \left(\frac{\partial v}{\partial x}\right)_y & \left(\frac{\partial v}{\partial y}\right)_x \end{pmatrix} \tag{12.35}$$

The following properties are easily derived from the definition.

$$\frac{\partial(u, v)}{\partial(x, y)} = -\frac{\partial(v, u)}{\partial(x, y)} = \frac{\partial(v, u)}{\partial(y, x)} = \left(\frac{\partial(x, y)}{\partial(u, v)}\right)^{-1}, \tag{12.36}$$

$$\frac{\partial(u, y)}{\partial(x, y)} = \left(\frac{\partial u}{\partial x}\right)_y, \tag{12.37}$$

$$\frac{\partial(u,v)}{\partial(x,y)} = \frac{\partial(u,v)}{\partial(t,s)}\frac{\partial(t,s)}{\partial(x,y)} = \frac{\dfrac{\partial(u,v)}{\partial(t,s)}}{\dfrac{\partial(x,y)}{\partial(t,s)}}. \tag{12.38}$$

We can accomplish the same result as above using the convenient properties of Jacobians.

$$\left(\frac{\partial V}{\partial T}\right)_p = \frac{\partial(V,p)}{\partial(T,p)} = \frac{\dfrac{\partial(V,p)}{\partial(T,V)}}{\dfrac{\partial(T,p)}{\partial(T,V)}} = -\frac{\left(\dfrac{\partial p}{\partial T}\right)_V}{\left(\dfrac{\partial p}{\partial V}\right)_T} = -\left(\frac{\partial p}{\partial T}\right)_V \left(\frac{\partial p}{\partial V}\right)_p. \tag{12.39}$$

This again leads to

$$\alpha = \frac{1}{V}\left(\frac{\partial V}{\partial T}\right)_p = -\frac{1}{V}\left(\frac{\partial p}{\partial T}\right)_V \left(\frac{\partial V}{\partial p}\right)_T. \tag{12.40}$$

12.3.3 Maxwell relations

Consider the total differential of the free energy as a function of the temperature, volume, and number of particles, $F(T,V,N)$.

$$\mathrm{d}F = -S\,\mathrm{d}T - p\,\mathrm{d}V + \mu\,\mathrm{d}N. \tag{12.41}$$

Now, compute the partial derivative of F with respect to T.

$$\frac{\partial F}{\partial T} = -S. \tag{12.42}$$

Now, we will differentiate again, this time with respect to V.

$$\frac{\partial^2 F}{\partial V \partial T} = -\frac{\partial S}{\partial V}. \tag{12.43}$$

We now repeat the differentiation again, this time differentiating first with respect to V and then with respect to T.

$$\frac{\partial^2 F}{\partial T \partial V} = -\frac{\partial p}{\partial T}. \tag{12.44}$$

But, the order of differentiation should not matter, so

$$\frac{\partial^2 F}{\partial T \partial V} = \frac{\partial^2 F}{\partial V \partial T}, \tag{12.45}$$

which gives that

$$\left(\frac{\partial S}{\partial V}\right)_{T,N} = \left(\frac{\partial p}{\partial T}\right)_{V,N} \tag{12.46}$$

This result is an example of a **Maxwell relation**. These are useful relationships between two derivatives of thermodynamic quantities that result from equality of mixed second derivatives of thermodynamic potentials. This relation was derived by differentiating the free energy with respect to V and T. We can derive two more using the free energy. First, by differentiating with respect to T and N,

$$-\left(\frac{\partial S}{\partial N}\right)_{T,V} = \left(\frac{\partial \mu}{\partial T}\right)_{V,N}, \tag{12.47}$$

and second by differentiating with respect to V and N,

$$-\left(\frac{\partial p}{\partial N}\right)_{T,V} = \left(\frac{\partial \mu}{\partial V}\right)_{T,N}. \tag{12.48}$$

Similar Maxwell relations may be derived from any Legendre transform of the energy (except the total Legendre transform, which is zero).

12.3.4 C_p, C_v, κ_T, and α

Let's use these tools to relate the susceptibilities. Let us state by writing the entropy as a function of T and V with constant N.

$$dS = \left(\frac{\partial S}{\partial T}\right)_V dT + \left(\frac{\partial S}{\partial V}\right)_T dV. \tag{12.49}$$

Differentiating with respect to T at constant p gives

$$\left(\frac{\partial S}{\partial T}\right)_p = \left(\frac{\partial S}{\partial T}\right)_V + \left(\frac{\partial S}{\partial V}\right)_T \left(\frac{\partial V}{\partial T}\right)_p \tag{12.50}$$

Using the relations

$$\frac{C_p}{T} = \left(\frac{\partial S}{\partial T}\right)_p \tag{12.51}$$

$$\frac{C_v}{T} = \left(\frac{\partial S}{\partial T}\right)_v \tag{12.52}$$

$$\left(\frac{\partial S}{\partial V}\right)_T = \left(\frac{\partial p}{\partial T}\right)_V, \tag{12.53}$$

the last being one of the Maxwell relations we derived above, we have

$$\frac{C_p}{T} = \frac{C_v}{T} + \left(\frac{\partial p}{\partial T}\right)_V \left(\frac{\partial V}{\partial T}\right)_p . \tag{12.54}$$

Using the triple product rule,

$$\left(\frac{\partial p}{\partial T}\right)_V = -\left(\frac{\partial p}{\partial V}\right)_T \left(\frac{\partial V}{\partial T}\right)_p . \tag{12.55}$$

Substituting this result into equation 12.54 yields

$$\frac{C_p}{T} = \frac{C_v}{T} - \left(\frac{\partial p}{\partial V}\right)_T \left(\left(\frac{\partial V}{\partial T}\right)_p\right)^2 \tag{12.56}$$

Using the definitions of α and κ_T,

$$\alpha = \frac{1}{V}\left(\frac{\partial V}{\partial T}\right)_p , \tag{12.57}$$

$$\kappa_T = -\frac{1}{V}\left(\frac{\partial V}{\partial p}\right)_T , \tag{12.58}$$

this is

$$\frac{C_p}{T} = \frac{C_v}{T} + V\frac{\alpha^2}{\kappa_T}, \tag{12.59}$$

As a result, we have

$$C_p = C_v + TV\frac{\alpha^2}{\kappa_T}. \tag{12.60}$$

We have already shown the C_v is positive, and κ_T is positive (left to be shown in homework), and the temperature and volume are both positive, so $C_p \geq C_v$.

12.4 Thermodynamic variables on a per-particle basis

It is often convenient to convert the extensive thermodynamic variables to intensive ones by dividing by the total number of molecules. For example, we can define $e = E/N$, $s = S/N$, $v = V/N$, $c_v = C_v/N$, and so on. The total differential of the entropy, in the case of constant N, is then

$$\mathrm{d}s = \frac{1}{T}\mathrm{d}e + \frac{p}{T}\mathrm{d}v. \tag{12.61}$$

That of the energy is

$$\mathrm{d}e = T\,\mathrm{d}s - p\,\mathrm{d}v, \tag{12.62}$$

and that of the free energy is

$$\mathrm{d}f = -s\,\mathrm{d}T - p\,\mathrm{d}v. \tag{12.63}$$

The Gibbs-Duhem relation is

$$\mathrm{d}\mu = -s\,\mathrm{d}T + v\,\mathrm{d}p. \tag{12.64}$$

The relation we just derived in the previous section is

$$c_p = c_v + Tv\,\frac{\alpha^2}{\kappa_T}. \tag{12.65}$$

12.5 Equations of state

The preceding was a demonstration of how different thermodynamic properties are related. We know, in a sense, what the thermodynamic properties are. They are expectations and parameters (specifically parameters that arise as Lagrange multipliers) that follow from a probability distribution of microstates that maximize the entropy. In order to know what the thermodynamic properties are, as expressed in terms of the expectations of the underlying probability distribution of microstates (the thermodynamic variables), we need to fully specify the energy, or the entropy, or a Legendre transform of either. To do so, we need to either

1. Have a microscopic model for the system where the microstates are defined and counted so that we may derive the probability distribution from which the thermodynamic variables may be calculated,
2. Measure the thermodynamic variables,
3. Invent a phenomenological model for the thermodynamic variables.

Let's say for a moment that we could specify an expression for the energy in terms of the entropy, volume, and number of particles.

$$E = E(S, V, N). \tag{12.66}$$

Recall the Euler relation as

$$E = \frac{\partial E}{\partial S}\,S + \frac{\partial E}{\partial V}\,V + \frac{\partial E}{\partial N}\,N. \tag{12.67}$$

If we know how each of the derivatives depend on the other independent variables, we will also have fully specified the energy. That is, if we know *all three* of

$$\frac{\partial E}{\partial S} = T(S,V,N) = T(s,v), \tag{12.68}$$

$$\frac{\partial E}{\partial V} = -p(S,V,N) = -p(s,v), \tag{12.69}$$

$$\frac{\partial E}{\partial N} = \mu(S,V,N) = \mu(s,v), \tag{12.70}$$

we will have all of the thermodynamic information. Each of the above are an example of an **equation of state**, which is a relationship between an intensive parameter and the extensive variables.

Without complete thermodynamic information, that is with only one or two of the equations of state, we can still get useful results by applying the thermodynamic relations we have derived. So, even having a single equation of state is useful. This is perhaps most evident by the most famous equation of state, which belongs to an ideal gas, $pV = Nk_BT$.

13 Solution thermodynamics

Having laid much of the groundwork of the theory of thermodynamics, we now turn our attention to a specific system, a solution containing solvent and solute molecules. Everything we have developed thus far is general; we need to specify all equations of state, or an energy, entropy, or Legendre transform thereof, to have all of the thermodynamic information. We will now work specifically with solutions and chemically reacting systems.

13.1 Conditions for equilibria of chemical reactions

While this section of the lecture material is about solution thermodynamics, we introduce chemical equilibria more generally here. This treatment holds for solutions, as well as gas and other phase reactions.

Let us assume that a mixture of chemical species N_i of species i. Then, the total differential of the energy is

$$\mathrm{d}E = T\,\mathrm{d}S - p\,\mathrm{d}V + \sum_i \mu_i \mathrm{d}N_i, \tag{13.1}$$

with the energy being

$$E = TS - pV + \sum_i \mu_i N_i, \tag{13.2}$$

where μ_i is the chemical potential of species i. Recall that the chemical potential is

$$\mu_i = \left(\frac{\partial E}{\partial N_i}\right)_{S,V,N_j} = -T\left(\frac{\partial S}{\partial N_i}\right)_{E,V,N_j}, \tag{13.3}$$

where $j \neq i$. Writing the Gibbs free energy as the Legendre transform of the energy, we have

$$G = E - TS + pV = \sum_i \mu_i N_i. \tag{13.4}$$

If we have a chemical reaction that proceeds in a system that is held at constant temperature and pressure, we have already worked out that the Gibbs free energy must be minimal at equilibrium. That means that

$$\frac{\mathrm{d}G}{\mathrm{d}N_i} = 0 \quad \forall i. \tag{13.5}$$

Writing this differential for $i = 1$ gives

$$\frac{\mathrm{d}G}{\mathrm{d}N_1} = \frac{\partial G}{\partial N_1}\frac{\partial N_1}{\partial N_1} + \frac{\partial G}{\partial N_2}\frac{\partial N_2}{\partial N_1} + \frac{\partial G}{\partial N_3}\frac{\partial N_3}{\partial N_1} + \cdots$$
$$= \mu_1 + \mu_2 \frac{\partial N_2}{\partial N_1} + \mu_3 \frac{\partial N_3}{\partial N_1} + \cdots = 0. \tag{13.6}$$

If we have a chemical reaction proceeding, the changes in the species are coupled. For every molecule of type 1 that is produced, ν_i/ν_1 molecules of type 2 are produced. Thus,

$$\frac{\partial N_i}{\partial N_1} = \frac{\nu_i}{\nu_1}. \tag{13.7}$$

Thus, we have

$$\frac{\mathrm{d}G}{\mathrm{d}N_1} = \sum_i \mu_i \frac{\nu_i}{\nu_1} = 0. \tag{13.8}$$

Multiplying by ν_1 gives the equilibrium condition for a chemical reaction,

$$\sum_i \nu_i \mu_i = 0. \tag{13.9}$$

This result is general. We have not specified the form of the chemical potential yet (we will in a moment for solutions). It holds for all reactions. Indexing the reactions with r, the equilibrium condition is

$$\sum_i \nu_{ri} \mu_i = 0 \quad \forall r. \tag{13.10}$$

13.2 A dilute solution of one solute

We will now work out the expression for the chemical potential of the components of a dilute solution. To start with, assume we have a total of N solvent molecules and no solute. The Gibbs free energy is

$$G = N\mu^0_{\mathrm{solv}}(T, p), \tag{13.11}$$

where I have explicitly shown the pressure- and temperature-dependence of the solvent chemical potential. I use the superscript 0 to denote that this is the chemical potential for pure solvent; we will soon see that its chemical potential changes as we add solute. Now, we will add one solute molecule to the solution. We define

by $\alpha(T,p,N)$ the amount that the Gibbs free energy changes as a result of adding a single solvent molecule. With this new molecule in the solution, we have

$$G = N\mu^0_{\text{solv}}(T,p) + \alpha(T,p,N). \tag{13.12}$$

Now, let's add another one. Naively, we would think that the result is

$$G = N\mu^0_{\text{solv}}(T,p) + 2\alpha(T,p,N). \tag{13.13}$$

There is a problem with this, though. Recall that the Gibbs free energy is related to a partition function by

$$G = -k_B T \ln \mathcal{Z}. \tag{13.14}$$

As we sum up each of the terms in the partition function $\mathcal{Z}$, we consider the configurations of the molecules. We could swap the first one we added with the second one we added. And configuration with this swap is identical to one without it. Therefore, we put too many terms, exactly twice too many terms, in the partition function by neglecting that we could swap out the molecules. So, we have a partition function that is twice as large as it should be as we have written the Gibbs free energy in gray above. So, the correct Gibbs free energy should is

$$G = N\mu^0_{\text{solv}}(T,p) + 2\alpha(T,p,N) + k_B T \ln 2. \tag{13.15}$$

We can keep adding particles until we get n of them.[14] The correction for overcounting is $n!$, since there are $n!$ different ways to swap the solute molecules we put in there. Therefore, the Gibbs free energy is

$$G = N\mu^0_{\text{solv}}(T,p) + n\alpha(T,p,N) + k_B T \ln n!. \tag{13.16}$$

Since n will typically be very large, we can use **Stirling's approximation** for the factorial,

$$n! \approx \sqrt{2\pi n}\left(\frac{n}{\mathrm{e}}\right)^n \left(1 + \mathcal{O}\left(n^{-1}\right)\right), \tag{13.17}$$

such that $\ln n! \approx n \ln n - n$. Applying this approximation,

$$\begin{aligned} G &= N\mu^0_{\text{solv}}(T,p) + n\alpha(T,p,N) + k_B T(n \ln n - n) \\ &= N\mu^0_{\text{solv}}(T,p) + n\left(\alpha(T,p,N) + k_B T \ln n - k_B T\right). \end{aligned} \tag{13.18}$$

Now, investigating the expression for the Gibbs free energy, we recall that it must be a first order homogeneous function of its extensive parameters, including the number

[14] I acknowledge that this introduces an extensive parameter that we are denoting with a lowercase symbol, but this is very widely done in the context of solutions, and we proceed with this notation, always remembering that n is extensive.

of solvent molecules N and the number of solute molecules n. This determines that $\alpha(T,p,N)$ must be of the form

$$\alpha(T,p,N) = \mu^0(T,p) - k_B T \ln N, \tag{13.19}$$

such that

$$\begin{aligned} G &= N\mu^0_{\text{solv}}(T,p) + n\left(\mu^0(T,p) - k_B T \ln N + k_B T(\ln n - 1)\right) \\ &= N\mu^0_{\text{solv}}(T,p) + n\left(\mu^0(T,p) + k_B T\left(\ln \frac{n}{N} - 1\right)\right) \end{aligned} \tag{13.20}$$

Note that we have neglected any interactions between solvent molecules. This means that we have assumed the solution is **dilute**. It we wished to include particle-particle interactions, we express their contributions to the Gibbs free energy, taking $\mu^0(T,p)$ as the first order contribution, and then $n^2\gamma(T,p)/2N$ as a second order contribution. (The contribution multiplying n^2 must be of the form $\gamma(T,p)/N$ to maintain first order homogeneity of the Gibbs free energy.) So, we can write the Gibbs free energy, to second order in the interactions of solute molecules, as

$$G = N\mu^0_{\text{solv}}(T,p) + n\left(\mu^0(T,p) + k_B T\left(\ln \frac{n}{N} - 1\right) + \frac{n}{2N}\gamma(T,p)\right). \tag{13.21}$$

Going forward, we will assume diluteness and ignore terms of order n^2 and higher in our expression for the Gibbs free energy and use equation (13.20).

13.3 A dilute solution with multiple solutes

The above analysis generalizes to multiple components. Equation (13.21) becomes

$$G = N\mu^0_{\text{solv}}(T,p) + \sum_i n_i \left(\mu^0_i(T,p) + k_B T\left(\ln \frac{n_i}{N} - 1\right) + \sum_j \frac{n_j}{2N}\gamma_{ij}(T,p)\right), \tag{13.22}$$

or, neglecting higher order terms,

$$G = N\mu^0_{\text{solv}}(T,p) + \sum_i n_i \left(\mu^0_i(T,p) + k_B T\left(\ln \frac{n_i}{N} - 1\right)\right), \tag{13.23}$$

Now that we have written down the Gibbs free energy, we can compute the chemical potential of species j as

$$\mu_j(T,p) = \left(\frac{\partial G}{\partial n_j}\right)_{T,p,N} = \mu^0_j(T,p) + k_B T \ln \frac{n_j}{N}. \tag{13.24}$$

We can also write the chemical potential of the solvent as

$$\mu_{\text{solv}} = \mu^0_{\text{solv}} - k_B T \sum_i \frac{n_i}{N}. \tag{13.25}$$

We define by x_i the **mole fraction** of species i as

$$x_i = \frac{n_i}{N + \sum_j n_j}. \tag{13.26}$$

In a dilute solution, there are many many more solvent molecules than solute, such that

$$N + \sum_j n_j \approx N, \tag{13.27}$$

and

$$x_i \approx \frac{n_i}{N}. \tag{13.28}$$

Then, we can write the Gibbs free energy, the solute chemical potential, and the solvent chemical potential respectively as

$$G = N\mu^0_{\text{solv}}(T,p) + \sum_i n_i \left(\mu^0_i(T,p) + k_B T(\ln x_i - 1)\right), \tag{13.29}$$

$$\mu_i(T,p) = \mu^0_i(T,p) + k_B T \ln x_i, \tag{13.30}$$

$$\mu_{\text{solv}} = \mu^0_{\text{solv}} - k_B T \sum_i x_i. \tag{13.31}$$

13.4 Osmotic pressure

Imagine we have two solutions in contact with each other. They could be separated by a semipermeable membrane through which solvent molecules may pass, but not solute. This would be the situation of a cell in buffer with closed ion channels. Water may pass through the membrane through porins, but not, for example, ions.

We can write down the Gibbs free energy of the respective solutions, labeled 1 and 2, as

$$\begin{aligned} G = {} & N_1\mu^0_{\text{solv}}(T,p_1) + n_1\left(\mu^0_1(T,p_1) + k_B T\left(\ln\frac{n_1}{N_1} - 1\right)\right) \\ & + N_2\mu^0_{\text{solv}}(T,p_2) + n_2\left(\mu^0_2(T,p_2) + k_B T\left(\ln\frac{n_2}{N_2} - 1\right)\right). \end{aligned} \tag{13.32}$$

Note that the temperatures of each solution are equal; they are in thermal equilibrium. By construction, there can be only equilibrium with respect to temperature (established by both having the same temperature) and solvent, but not solute, since solute cannot pass between phases. So, we must have, at equilibrium,

$$\begin{aligned}\frac{\mathrm{d}G}{\mathrm{d}N_1} &= \left(\frac{\partial G}{\partial N_1}\right)_{T,p}\frac{\partial N_1}{\partial N_1} + \left(\frac{\partial G}{\partial N_2}\right)_{T,p}\frac{\partial N_2}{\partial N_1} \\ &= \left(\frac{\partial G}{\partial N_1}\right)_{T,p} - \left(\frac{\partial G}{\partial N_2}\right)_{T,p} = 0, \end{aligned} \tag{13.33}$$

where we have used $\partial N_2/\partial N_1 = -1$, since every solvent molecule that is lost from solution 2 goes into solution 1. The partial derivatives are computed as

$$\frac{\partial G}{\partial N_1} = \mu^0_{\text{solv}}(T,p_1) - k_B T\frac{n_1}{N} = \mu^0_{\text{solv}}(T,p_1) - k_B T x_1 = 0 \tag{13.34}$$

$$\frac{\partial G}{\partial N_2} = \mu^0_{\text{solv}}(T,p_2) - k_B T\frac{n_2}{N} = \mu^0_{\text{solv}}(T,p_2) - k_B T x_2 = 0. \tag{13.35}$$

By equation 13.33, these must be equal, giving

$$\mu^0_{\text{solv}}(T,p_1) - k_B T x_1 = \mu^0_{\text{solv}}(T,p_2) - k_B T x_2, \tag{13.36}$$

which we note is equivalent to equality of chemical potential of solvent. In fact, for *any* species that may exchange between two solutions, equality of chemical potential of that species in each of the two solutions is an equilibrium condition, regardless of diluteness of the solution, as you will show in homework.

Using the expression for equality of chemical potential of solvent, we have that

$$\mu^0_{\text{solv}}(T,p_2) - \mu^0_{\text{solv}}(T,p_1) = k_B T(x_2 - x_1). \tag{13.37}$$

The left hand side is the difference in chemical potential of pure solvent for different pressures. For dilute solutions, the pressure difference $\Pi \equiv p_2 - p_1$ is often small, so we can expand $\mu^0_{\text{solv}}(T,p_2)$ to linear order in Π.

$$\mu^0_{\text{solv}}(T,p_2) \approx \mu^0_{\text{solv}}(T,p_1) + \left(\frac{\partial \mu^0_{\text{solv}}}{\partial p}\right)_{T,N}\Pi. \tag{13.38}$$

Using a Maxwell relation derived from the Gibbs free energy,

$$\left(\frac{\partial \mu^0_{\text{solv}}}{\partial p}\right)_{T,N} = \left(\frac{\partial V}{\partial N}\right)_{T,p} \tag{13.39}$$

This latter derivative is called the **partial molar volume**, which is the amount that the volume changes as a result of adding more molecules. For a pure substance,

which is what the case here, coming from an expansion of the pure solvent chemical potential, it is the inverse of the number density of solvent, $1/\rho_{\text{solv}}$. If the volume of solvent is V, then $\rho_{\text{solv}} = N/V$. Thus, we have

$$\mu^0_{\text{solv}}(T,p_2) - \mu^0_{\text{solv}}(T,p_1) \approx \frac{\Pi}{\rho_{\text{solv}}} = \frac{V}{N}\,\Pi. \tag{13.40}$$

Rearranging the equilibrium condition using this expression, we have

$$\Pi = \frac{Nk_BT}{V}(x_2 - x_1). \tag{13.41}$$

This difference in pressure Π between one solution and the other is called **osmotic pressure**. The above relation, valid for dilute solutions, is called the **van't Hoff formula** (not to be confused with the van't Hoff equation that related chemical equilibrium constants to enthalpy) or the **Morse equation**.

13.5 Chemical equilibria and the law of mass action

Now that we have written down expressions for the chemical potentials of solutes, we can write down the equilibrium conditions for chemical reactions in dilute solutions. Recall that the equilibrium condition for a chemical reaction is

$$\sum_i \nu_i\,\mu_i = 0. \tag{13.42}$$

Substituting in the expression for solute chemical potentials, this is

$$\sum_i \nu_i \left(\mu_i^0(T,p) + k_BT\ln x_i\right) = 0. \tag{13.43}$$

We can rearrange this to be

$$\exp\left[-\sum_i \nu_i\,\beta\,\mu_i^0(T,p)\right] = \prod_i x_i^{\nu_i}. \tag{13.44}$$

The term on the left hand side is referred to as the **equilibrium constant**, usually denoted as K, and is a function of temperature and pressure. The resulting equation,

$$K \equiv \exp\left[-\sum_i \nu_i\,\beta\,\mu_i^0(T,p)\right] = \prod_i x_i^{\nu_i}, \tag{13.45}$$

is referred to as **the law of mass action**.

As an example, consider a system with solute A, B, and AB, where the chemical reaction $\mathrm{AB} \rightleftharpoons \mathrm{A} + \mathrm{B}$ may occur. Then, we have $\nu_{\mathrm{A}} = \nu_{\mathrm{B}} = 1$ and $\nu_{\mathrm{AB}} = -1$. The resulting equilibrium condition is

$$K_{\mathrm{d}} \equiv \exp\left[-\frac{\mu^0_{\mathrm{A}} + \mu^0_{\mathrm{B}} - \mu^0_{\mathrm{AB}}}{k_B T}\right] = \frac{x_{\mathrm{A}}\, x_{\mathrm{B}}}{x_{\mathrm{AB}}}. \tag{13.46}$$

Note that in this special case, I have labeled the equilibrium constant as K_{d}, which is referred to as a **dissociation constant**, which is a term applied to an equilibrium constant describing a reaction involving the unbinding of two species.

Dissociation constants are often reported as

$$K_{\mathrm{d}} = \frac{c_{\mathrm{A}}\, c_{\mathrm{B}}}{c_{\mathrm{AB}}}, \tag{13.47}$$

giving a dissociation constant in units of concentration. In this case, the expression relating the equilibrium constant to an exponentiation of differences of the pure-species chemical potentials is nonsensical because of a unit mismatch. Therefore, if the K_d is to be reported in units of concentration, it we must have

$$K_{\mathrm{d}} = \rho_{\mathrm{solv}} \exp\left[-\frac{\mu^0_{\mathrm{A}} + \mu^0_{\mathrm{B}} - \mu^0_{\mathrm{AB}}}{k_B T}\right], \tag{13.48}$$

where ρ_{solv} is the number density of the solvent.

13.6 Nonideal solutions and activities

So far, we have been working with dilute solutions wherein the chemical potential of solute is given by

$$\mu_i(T,p) = \mu^0_i(T,p) + k_B T \ln x_i. \tag{13.49}$$

Such solutions are called **ideal solutions**. As concentrations rise, the chemical potential deviates from this expression. To handle nonidealities, we introduce a drop-in replacement for the mole fraction called **activity** a_i to maintain the same functional form of the chemical potential.

$$\mu_i(T,p) = \mu^0_i(T,p) + k_B T \ln a_i(T,p,\mathbf{x}). \tag{13.50}$$

Note that in general the activity is a function of temperature, pressure, and the mole fractions of all species in solution. The activities are typically determined experimentally or have a phenomenological functional form. It is also common to define $a_i = \gamma_i x_i$, where γ_i is the **activity coefficient**, which is unity for an ideal solution.

In all of the analysis we have done in solution thermodynamics, we can drop in a_i (or $\gamma_i x_i$) for x_i to handle the nonideal conditions. The caveat is that the nonidealities may introduce thermodynamic instabilities that are not present for ideal solutions.

14 Electrochemistry

Many solutions in and out of the living world contain charged species. These charged species, through electrostatic interactions, contribute to the total energy of a system. To derive their contributions, we can take the usual approach using a generalized potential as in Section 11.5.

14.1 Charged species in solution

We define Q (not to be confused with the heat Q) to be the total charge of charged species in solution, which can in general be a function of position. This is an extensive variable given by

$$Q = \sum_i z_i \, e \, N_i, \tag{14.1}$$

where z_i is the **valence**, the number of charges per molecule or ion, of species i, e is the elementary charge of a proton (1.602×10^{-19} Coulombs), and N_i is the number of molecules of species i. We define the **electrostatic potential** ψ as

$$\psi = T \left(\frac{\partial S}{\partial Q} \right)_{E,V,N}. \tag{14.2}$$

All charged species experience this potential, which is induced by electrodes applying a potential or by presence of other charges. The electrostatic potential, like the distribution of charges, is in general dependent on space. With these definitions, we can write the total differential of the energy,

$$\begin{aligned} \mathrm{d}E &= T\,\mathrm{d}S - p\,\mathrm{d}V + \sum_i \mu_i \,\mathrm{d}N_i + \psi \,\mathrm{d}Q \\ &= T\,\mathrm{d}S - p\,\mathrm{d}V + \sum_i (\mu_i + z_i \, e \, \psi) \,\mathrm{d}N_i, \end{aligned} \tag{14.3}$$

where we have used the fact that

$$\mathrm{d}Q = e \sum_i z_i \,\mathrm{d}N_i. \tag{14.4}$$

The parenthetical term is referred to as the **electrochemical potential** for species i, which we will denote by $\bar{\mu}_i$, given by

$$\bar{\mu}_i = \mu_i + z_i \, e \, \psi. \tag{14.5}$$

We can perform a Legendre transform of the energy to obtain the Gibbs free energy, which is useful to consider for a system at constant temperature and pressure.

$$\begin{aligned} \mathrm{d}G &= -S\,\mathrm{d}T + V\,\mathrm{d}p + \sum_i \bar{\mu}_i\,\mathrm{d}N_i \\ &= -S\,\mathrm{d}T + V\,\mathrm{d}p + \sum_i (\mu_i + z_i\,e\,\psi)\,\mathrm{d}N_i. \end{aligned} \tag{14.6}$$

We can use thermodynamic considerations to work out spatial equilibrium charge distributions in electrolytic solutions. Imagine a small slice of solution containing electrolytes positioned at x next to another small slice positioned at $x + \Delta x$. At constant temperature and pressure, the equilibrium condition ($\mathrm{d}G = 0$) is that the electrochemical potentials of the respective slices are equal (as you will show in homework),

$$\begin{aligned} \bar{\mu}_i(x + \Delta x) &= \mu_i(x + \Delta x) + z_i\,e\,\psi(x + \Delta x) \\ &= \bar{\mu}_i(x) = \mu_i(x) + z_i\,e\,\psi(x) \quad \forall i. \end{aligned} \tag{14.7}$$

Rearranging, we have

$$\mu_i(x + \Delta x) - \mu_i(x) = -z_i\,e(\psi(x + \Delta x) - \psi(x)). \tag{14.8}$$

Dividing both sides by Δx and taking the limit where $\Delta x \to 0$ gives

$$\frac{\mathrm{d}\mu_i}{\mathrm{d}x} = -z_i\,e\,\frac{\mathrm{d}\psi}{\mathrm{d}x}. \tag{14.9}$$

This generalizes to two or three dimensions as

$$\nabla_{\mathbf{x}}\mu_i = -z_i\,e\,\nabla_{\mathbf{x}}\psi. \tag{14.10}$$

If we have a dilute solution, then $\mu_i = \mu_i^0 + k_B T \ln x_i$, and we have (considering the one-dimensional case for simplicity, where the mole fractions x_i are not to be confused with the spatial coordinate x),

$$k_B T\,\frac{\mathrm{d}\ln x_i}{\mathrm{d}x} = -z_i\,e\,\frac{\mathrm{d}\psi}{\mathrm{d}x}. \tag{14.11}$$

Note also that we can consider any two positions in a solution that is in thermal equilibrium. Since every differential slice of solution is in equilibrium with its neighbors, any two differential slices are in equilibrium with each other. Therefore, we can consider two positions, x_1 and x_2, and thermodynamic equilibrium enforces that the electrochemical potentials are equal, giving

$$\bar{\mu}_i(x_1) = \mu_i(x_1) + z_i\,e\,\psi(x_1) = \bar{\mu}_i(x_2) = \mu_i(x_2) + z_i\,e\,\psi(x_2). \tag{14.12}$$

Assuming a dilute solution, this is

$$k_B T \ln x_i(x_1) + z_i e \psi(x_1) = k_B T \ln x_i(x_2) + z_i e \psi(x_2), \tag{14.13}$$

which can be rearranged to give

$$\ln \frac{x_i(x_2)}{x_i(x_1)} = -\beta z_i e(\psi(x_2) - \psi(x_1)). \tag{14.14}$$

This is sometimes referred to as the **Nernst equation**, though in chemistry the Nernst equation is more often taken to be as it is defined in section 14.4.

14.2 Charge neutrality

In most calculations involving electrochemistry, we assume that the number of positive charges (e.g., one million for a million sodium ions and two million for a million magnesium ions) and negative charges are equal. This is called **charge neutrality**. To understand from whence this assumption comes, consider what would happen if we had an imbalance of positive and negative ions, say with more positive ions than negative. The electrostatic potential of a charged object is given by $V = Q/C$, where Q is the total charge and C is its capacitance. Water is quite effective at conducting current, and the capacitance of spherical conducting object is approximately $C = 4\pi\varepsilon_0 R$, where R is the radius of the sphere. For a giant sphere, say an entire meter in diameter, the capacitance is about 10^{-10} Farads, since $\varepsilon_0 = 8.85 \times 10^{-12}$ F/m. One Farad is one Coulomb per volt, and there are about 6×10^{18} individual charges per Coulomb. So, in order to have an electostatic potential of one volt in a giant four cubic meter volume (4000 liters), we would need to have $Q = VC = 10^{-10}$ Coulombs of charge, or a charge imbalance of 10^8 charges. At nanomolar concentrations of monovalent ions, say NaCl, we have a total of about 2.5×10^{18} each of positive and negative ions. So, even at very low concentrations, the charge imbalance even at high voltages, is totally negligible compared to total concentration of charged species. Thus, we generally have

$$\text{total number of positive charges} = \text{total number of negative charges} \tag{14.15}$$

in a solution.

14.3 The Donnan potential

We previously considered two solvent phases in contact in Section 13.4. In that case, we did not explicitly take into account charges of the solute. Let us assume that solvent may pass between the two phases, as may small ions, like sodium and chloride, but not a charged macromolecular solute. Recall that in our analysis of uncharged

species, an equilibrium condition is equality of chemical potential of an exchangeable species in the two phases. As you will show in your homework, in the case of charged exchangeable species, the equality of *electro*chemical potential is an equilibrium condition.

Considering first the solvent, we can find using the same analysis of Section 13.4 that the pressure difference between the two phases is the osmotic pressure

$$\Pi = \rho_{\text{solv}} k_B T (x_2 - x_1), \tag{14.16}$$

where x_1 and x_2 are the total solute concentrations in the respective phases.

Now we will consider the solutes. For purposes of discussion, let's say we have a mole fraction $x_{+,1}$ of positively charged monovalent ions and $x_{-,1}$ negatively charged monovalent ions in one phase, and $x_{+,2}$ and $x_{-,2}$ respectively in the other phase. The other phase also has a mole fraction x_2 of a macromolecule that has charge z.

If we consider positively charged solute, equality of electrochemical potential gives

$$\bar{\mu}_{+,1} = \bar{\mu}_{+,2}, \tag{14.17}$$

which for a dilute solution is

$$\mu^0_{+,1}(T,p) + k_B T \ln x_{+,1} + z_+ e \psi_1 = \mu^0_{+,2}(T, p + \Pi) + k_B T \ln x_{+,2} + z_+ e \psi_2. \tag{14.18}$$

Here, since the positive ion is monovalent, $z_+ = 1$. As when we treated osmotic pressure,

$$\mu^0_{+,2}(T, p + \Pi) \approx \mu^0_{+,1}(T,p) + \Pi / \rho_+, \tag{14.19}$$

where ρ_+ is the number density of *pure* solute. Note that this is a bit of a strange number density, since it is the number density of pure solute *under the conditions in which it is in solution*. So, it is not a realizable number density. Then,

$$e(\psi_2 - \psi_1) = -\frac{\Pi}{\rho_+} - k_B T \ln \frac{x_{+,2}}{x_{+,1}}. \tag{14.20}$$

Dividing through by e gives

$$\psi_2 - \psi_1 = -\frac{\Pi}{e\rho_+} - \frac{k_B T}{e} \ln \frac{x_{+,2}}{x_{+,1}}. \tag{14.21}$$

Similarly,

$$\psi_2 - \psi_1 = -\frac{\Pi}{e\rho_-} + \frac{k_B T}{e} \ln \frac{x_{-,2}}{x_{-,1}}. \tag{14.22}$$

For dilute solutions, these equations are

$$\psi_2 - \psi_1 = -\frac{\rho_{\text{solv}}(x_2 - x_1)}{e\rho_+} - \frac{k_B T}{e} \ln \frac{x_{+,2}}{x_{+,1}} \tag{14.23}$$

$$\psi_2 - \psi_1 = -\frac{\rho_{\text{solv}}(x_2 - x_1)}{e\rho_-} + \frac{k_B T}{e} \ln \frac{x_{-,2}}{x_{-,1}}, \tag{14.24}$$

where x_1 and x_2 are respectively the total mole fraction of all solutes in the two solutions.

The fact that an imbalance of ions on either side of a membrane gives a potential difference across a membrane is called the **Donnan effect** and the potential difference, $\psi_2 - \psi_1$ is called the **Donnan potential**. The Donnan potential is a major contributor to the potential across cell membranes and therefore has important consequences in cell physiology, most notably in nerve cells.

To solve for the Donnan potential, we need to solve for the four mole fractions $x_{+,1}, x_{-,1}, x_{+,2}$, and $x_{-,2}$. We need to know the partial molar volumes of pure species, ρ_{solv}, ρ_+, and ρ_-. However, the osmotic pressure terms that contain these constants are typically negligible compared to the other terms comprising the Donnan potential. In that case, we have

$$\ln \frac{x_{+,2}}{x_{+,1}} = \ln \frac{x_{-,1}}{x_{-,2}}, \tag{14.25}$$

or

$$\frac{x_{+,2}}{x_{+,1}} = \frac{x_{-,1}}{x_{-,2}}. \tag{14.26}$$

Now, also by charge neutrality,

$$x_{+,1} = x_{-,1}, \tag{14.27}$$

$$x_{+,2} - x_{-,2} + z x_2 = 0. \tag{14.28}$$

Clearly, if $x_2 = 0$, we have $x_{+,1} = x_{-,1} = x_{+,2} = x_{-,2}$, but the presence of the charged macromolecule that cannot cross phases tips the balance, and the concentration of ions on either side of the membrane will be unequal. To work out the concentrations of the species, and therefore the Donnan potential, we can use equations (14.26)-(14.28), plus another expression, possibly a conservation of charged ion $x_{+,1} + x_{+,2} = x_+^0$.

14.4 Electrochemistry of reactions

We have already worked out the equilibrium condition for a chemical reaction in the absence of an electrostatic field (that is for $\psi = 0$). Now, consider a reaction that

is occurring producing charged species (including electrons) in the presence of an electric field. A subclass of these reactions of great biological importance are **redox reactions**, in which a species accepts electrons (is reduced) or donates electrons (is oxidized). A classic example is the oxidation of vitamin C,

$$\mathrm{C_6H_8O_6} \rightleftharpoons \mathrm{C_6H_6O_6} + 2\,\mathrm{H^+} + 2\,\mathrm{e^-}. \tag{14.29}$$

Using precisely the same arguments as in Section 13.1, we can derive the equilibrium conditions for a redox reaction to be

$$\sum_i \nu_i \bar{\mu}_i = 0. \tag{14.30}$$

For a dilute solution this is

$$\sum_i \nu_i \left(\mu_i^0 + k_B T \ln x_i + z_i e\, \psi\right) = 0. \tag{14.31}$$

Rearranging, we have

$$\left[\mathrm{e}^{-\beta \sum_i \nu_i \mu_i^0}\right] \mathrm{e}^{-\beta e \psi \sum_i \nu_i z_i} = \prod_i x_i^{\nu_i}. \tag{14.32}$$

We recognize the bracketed as the equilibrium constant and the right hand side as the expression we are used to seeing from the law of mass action. The unbracketed term on the left hand side serves as an adjustment to the equilibrium constant due to the presence of an electrostatic potential ψ. Note that the correction is unity in the absence of an electrostatic potential ($\psi = 0$) and/or in the absence of charged species $z_i = 0\ \forall i$).

When written in a different form,

$$\mathcal{E} = \mathcal{E}^0 - \frac{k_B T}{n} \ln \prod_i x_i^{\nu_i}, \tag{14.33}$$

where

$$n = \sum_i \nu_i z_i \tag{14.34}$$

is the directional total number of electrons that that get transferred in the reaction, and

$$\mathcal{E} = \frac{\psi}{n} \tag{14.35}$$

is the **electromotive force**, so named because it describes how the presence of an electrostatic field drives flow of charges in a reaction. The quantity

$$\mathcal{E}^0 = \frac{1}{n} \sum_i \nu_i \mu_i^0 \tag{14.36}$$

is (confusingly, in my opinion) called the standard reduction potential in the context of redox reactions. I prefer simply to think of it according to its equation. If K is the equilibrium constant in the absence of an electrostatic field, then

$$\mathcal{E}^0 = \frac{k_B T}{n} \ln K. \tag{14.37}$$

Equation 14.33 is called the Nernst equation by chemists. Note that each term in the Nernst equation has dimension of energy per charge, most commonly reported in volts.

Part III

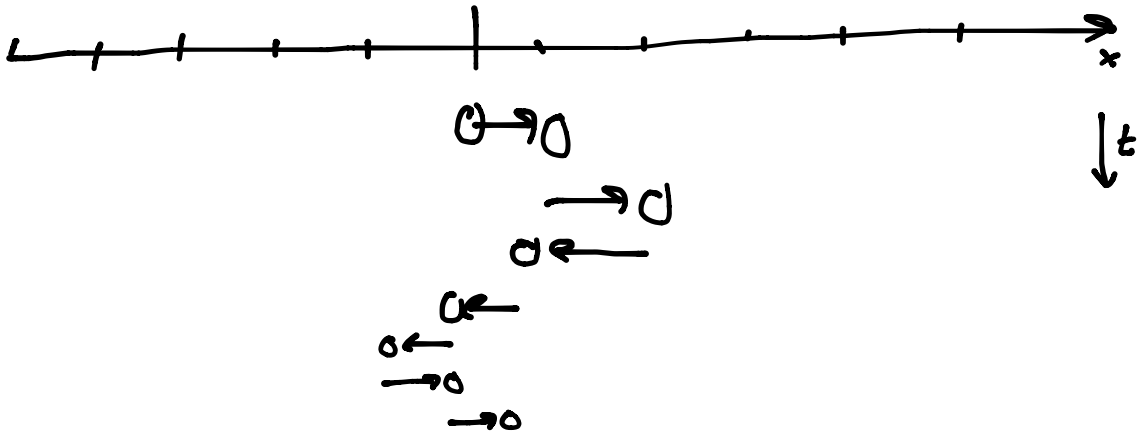
Kinetics and thermodynamics together

15 Transport by thermal diffusion

The typed lecture notes on diffusion are forthcoming. Please see the following handwritten notes for now.

Now, let's consider the particulate perspective.

Say I have a particle and every time period of length τ, it gets nudged randomly by solvent a distance l_{step} either right or left.



In this example, in time 7τ, the particle took $N_r = 4$ steps to the right and $N_l = 3$ steps to the left. So, its position after time 7τ is $+l_{step}$.

This process of taking a series of steps in random directions is called a random walk.

Question: For a random walk of N steps, what is the mean displacement? (Note: each step is of length l_{step}, and it takes time τ to take a step.)

By "displacement," we mean the distance from the end point of the random walk from the start.

Let r_i be the displacement of a single step i in units of l_{step}. For our 1D random walk, $r_i = +1, -1$ with equal probability.

Therefore, the total displacement (in units of l_{step}) is

$$\delta = \sum_{i=1}^{N} r_i$$

The mean displacement is

$$\langle \delta \rangle = \left\langle \sum_{i=1}^{N} r_i \right\rangle = \sum_{i=1}^{N} \langle r_i \rangle = N \langle r_i \rangle$$

Now, $\langle r_i \rangle = P_r(+1) + P_l(-1) = \frac{1}{2} - \frac{1}{2} = 0 \Rightarrow \boxed{\langle \delta \rangle = 0}$

(P_r: prob of stepping right; P_l: prob of stepping left)

Question: What is the mean square displacement, $\langle \delta^2 \rangle$?

We take the same approach:

$$\langle \delta^2 \rangle = \left\langle \left(\sum_{i=1}^{N} r_i \right)^2 \right\rangle = \sum_{i=1}^{N} \langle r_i^2 \rangle + \sum_{i=1}^{N} \sum_{\substack{j=1 \\ j \neq i}}^{N} \langle r_i r_j \rangle$$

$$= N \langle r_i^2 \rangle + N(N-1) \langle r_i r_j \rangle$$

What is $\langle r_i^2 \rangle$ (in units of l_{step}^2)?

$$\langle r_i^2 \rangle = \frac{(+1)^2 + (-1)^2}{2} = 1$$

For $i \neq j$, we can calculate $\langle r_i r_j \rangle$:

r_i	r_j	$r_i r_j$	probability
+1	+1	1	$P_r P_r = \frac{1}{4}$
+1	-1	-1	$P_r P_l = \frac{1}{4}$
-1	+1	-1	$P_l P_r = \frac{1}{4}$
-1	-1	1	$P_l P_l = \frac{1}{4}$

$\Rightarrow \langle r_i r_j \rangle = \frac{1}{4} - \frac{1}{4} - \frac{1}{4} + \frac{1}{4} = 0.$

Thus, we have $\boxed{\langle \delta^2 \rangle = N}$

So, on average, a random walk starts where it begins, but makes an excursion of $l_{step}\sqrt{\langle \delta^2 \rangle} = \sqrt{N}\, l_{step}$.

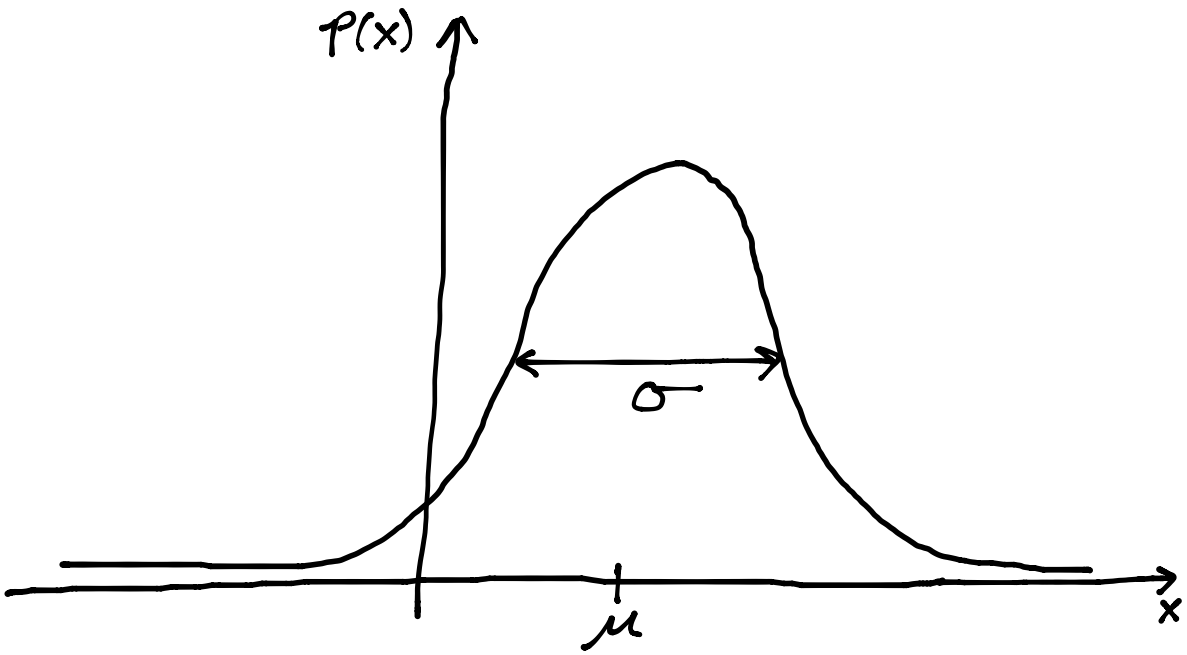
Question: After N steps, what is the probability that the displacement is δ?

We could just approximate $P(\delta|N)$ as a Gaussian, since we know the first two moments, $\langle \delta \rangle$ and $\langle \delta^2 \rangle$.

A Gaussian distribution is given (in 1D) by

$$P(x) = \left(\frac{1}{2\pi\sigma^2}\right)^{\frac{1}{2}} e^{-(x-\mu)^2/2\sigma^2},$$

where $\mu = \langle x \rangle$ and $\sigma^2 = \langle x^2 \rangle - \langle x \rangle^2$. μ is the mean and σ^2 is the variance.



So, taking $P(\delta|N)$ to be Gaussian, with $\mu = \langle \delta \rangle = 0$ and $\sigma^2 = \langle x^2 \rangle = N^2$,

$$\boxed{P(\delta|N) \approx \frac{1}{\sqrt{2\pi N}} e^{-\delta^2/2N^2}.}$$

We can also do a more careful analysis:

$$P(\delta|N) = \frac{\#\text{ of ways to take } N_r \text{ of } N \text{ steps to the right}}{\text{total number of 1D random walks of length } N}$$

$$\delta = N_r - N_l = N_r - (N - N_r) = 2N_r - N.$$

We already know how to compute the numerator:

$$P(\delta|N) \propto \frac{N!}{N_r!(N-N_r)!} = \frac{N!}{\left(\frac{N}{2}+\frac{\delta}{2}\right)!\left(\frac{N}{2}-\frac{\delta}{2}\right)!}$$

Therefore, we have,

$$\ln P(\delta|N) = \text{const} + \ln N! - \ln\left(\frac{N}{2}+\frac{\delta}{2}\right)! - \ln\left(\frac{N}{2}-\frac{\delta}{2}\right)!$$

For large N, we can use Stirling's approximation:

$$\ln P(\delta|N) \simeq \text{const} + N\ln N - N - \left(\frac{N}{2}+\frac{\delta}{2}\right)\ln\left(\frac{N}{2}+\frac{\delta}{2}\right) - \frac{N}{2} - \frac{\delta}{2}$$
$$- \left(\frac{N}{2}-\frac{\delta}{2}\right)\ln\left(\frac{N}{2}-\frac{\delta}{2}\right) - \frac{N}{2} + \frac{\delta}{2}$$

$$= \text{const.} + N\ln N - \frac{N}{2}\ln\left[\left(\frac{N}{2}+\frac{\delta}{2}\right)\left(\frac{N}{2}-\frac{\delta}{2}\right)\right] + \frac{\delta}{2}\ln\frac{\frac{N}{2}-\frac{\delta}{2}}{\frac{N}{2}+\frac{\delta}{2}}$$

$$= \text{const.} + N\ln N - \frac{N}{2}\ln\left(\frac{N^2}{4}-\frac{\delta^2}{4}\right) + \frac{\delta}{2}\ln\frac{1-\delta/N}{1+\delta/N}$$

$$= \underbrace{\text{const.} + N\ln N - \frac{N}{2}\ln\frac{N^2}{4}}_{\text{call this } \ln a} - \frac{N}{2}\ln\left(1-\frac{\delta^2}{N^2}\right) + \frac{\delta}{2}\ln\frac{1-\delta/N}{1+\delta/N}$$

Now, if we consider $\delta \ll N$, $\frac{\delta}{N} \ll 1$, and we can do Taylor expansions about $\frac{\delta}{N} = 0$.

$$\ln\left(1-\frac{\delta^2}{N^2}\right) \simeq -\frac{\delta^2}{N^2} \quad \text{and} \quad \ln\frac{1-\delta/N}{1+\delta/N} \simeq -2\frac{\delta}{N}$$

Thus, we have,

$$\ln P(\delta|N) \simeq \ln a - \frac{N}{2}\left(-\frac{\delta^2}{N^2}\right) + \frac{\delta}{2}\left(-2\frac{\delta}{N}\right) = \ln a - \frac{\delta^2}{2N}.$$

So, $P(\delta|N) = a\, e^{-\delta^2/2N}$.

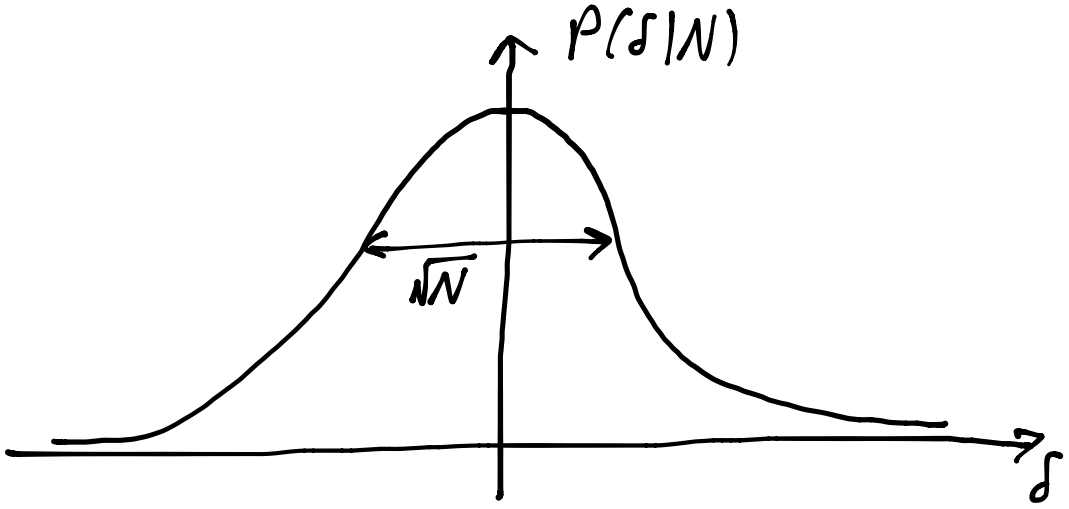
To get a, we note that $\int_{-\infty}^{\infty} d\delta\, P(\delta|N) = 1$, which is the normalization condition.

This integral is given in table 17.1.1 in your book.

We get:

$$P(\delta|N) = \frac{1}{\sqrt{2\pi N}}\, e^{-\delta^2/2N}$$

This is a Gaussian distribution. This particular one has mean of 0 and variance of N.



We can relate δ and N to physical distance and time.

$$x = \delta\, l_{step} \quad \text{and} \quad t = N\tau$$

So, $$\boxed{P(x,t) = \frac{1}{\sqrt{2\pi\, t/\tau}\, l_{step}} e^{-\tau x^2 / 2 l_{step}^2 t}.}$$

Now, let's compare the particulate and continuum

It stands to reason that $c(x,t) \propto P(x,t)$, or $c(x,t) = n_0 P(x,t)$, where n_0 is the total # of particles, since $n_0 = \int_{-\infty}^{\infty} dx\, c(x,t) = n_0 \underbrace{\int_{-\infty}^{\infty} dx\, P(x,t)}_{=1}$

Let's try plugging this into Fick's 2nd Law. First, compute derivs:

$$\frac{\partial c}{\partial t} = \frac{1}{2}\left(\frac{\tau x^2}{l_{step}^2 t^2} - \frac{1}{t}\right) c \quad ; \quad \frac{\partial^2 c}{\partial x^2} = \frac{\tau}{l_{step}^2}\left(\frac{\tau x^2}{l_{step}^2 t^2} - \frac{1}{t}\right) c.$$

$$\Rightarrow \frac{\partial c}{\partial t} = \frac{2\tau}{l_{step}^2} \frac{\partial^2 c}{\partial x^2}.$$

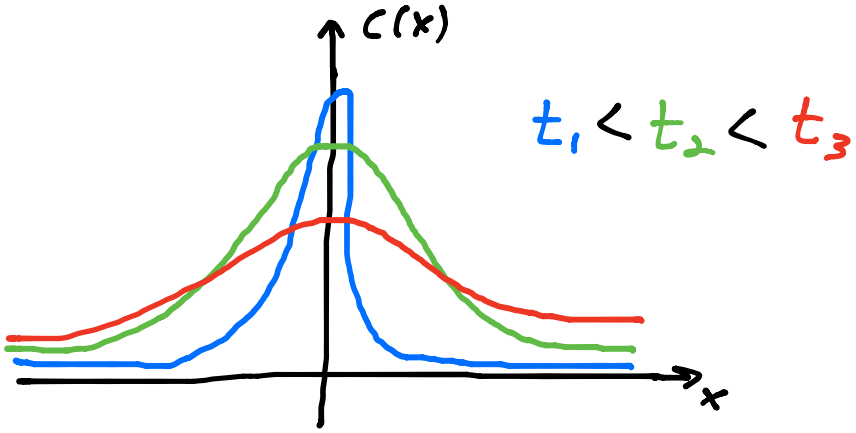
Thus, our expression for c satisfies Fick's 2nd Law with $\boxed{D = \frac{l_{step}^2}{2\tau}}$.

Now that we have a relationship between D, l_{step}, and τ, we have: $\langle x^2 \rangle = l_{step}^2 \langle \delta^2 \rangle = l_{step}^2 N = \frac{l_{step}^2}{\tau} t = 2Dt.$

So, the mean square displacement is <u>linear in time</u>.

Using $C = n_0 P(x,t)$, we can plug in our expression for D:

$$\boxed{C = n_0 \left(\frac{1}{4\pi Dt}\right)^{1/2} e^{-x^2/4Dt}}$$



Extending these results to 2D or 3D is trivial.

$\underset{\sim}{r} \equiv (x,y,z) \Rightarrow \underset{\sim}{r}^2 = x^2 + y^2 + z^2$

$\langle \underset{\sim}{r}^2 \rangle = \langle x^2 \rangle + \langle y^2 \rangle + \langle z^2 \rangle = 6Dt.$

$P(\underset{\sim}{r},t) = P(x,t)P(y,t)P(z,t)$

So, $P(\underset{\sim}{r},t) = \left(\frac{1}{4\pi Dt}\right)^{\frac{3}{2}} e^{-\underset{\sim}{r}^2/4Dt}$ $\quad (\underset{\sim}{r}^2 = x^2 + y^2 + z^2)$

$$\Rightarrow \boxed{C(r,t) = n_0 \left(\frac{1}{4\pi Dt}\right)^{\frac{3}{2}} e^{-\underset{\sim}{r}^2/4Dt}} \quad \text{in 3D.}$$

Question: Can we find an expression for D?

Imagine we have diffusing particles and we subject them to a force field, like gravity in a centrifuge. Call this force F. If the particle is dragged through the fluid, it experiences frictional drag. By Newton's second law, this drag must balance the force:

$$F(x) = f v(x)$$

(v: particle velocity (in x-dir.); f: friction factor)

The net flux of particles in the x-direction is $J_F = cv$, where the subscript "F" denotes flux due to the force field.

Now, at equilibrium, there is no net flux, so

$$J_F + J_D = 0,$$

where $J_D = -D\frac{dc}{dx}$ is the diffusive flux.

Therefore, we have $vc - D\frac{dc}{dx} = \frac{F}{f}c - D\frac{dc}{dx} = 0$.

So, $\frac{dc}{dx} = \frac{Fc}{fD}$.

But, we also know that at equilibrium, by the Boltzmann rule, the probability that a particle is at position x is

$$P(x) = \frac{1}{Q} e^{-U(x)/k_B T}.$$

We have $c(x) \propto P(x)$, or $c = \text{const} \cdot e^{-U(x)/k_B T}$.

Therefore,

$$\frac{dc}{dx} = \text{const} \cdot e^{-U(x)/k_B T}\left(-\frac{1}{k_B T}\frac{dU}{dx}\right) = \frac{F}{k_B T}c.$$

Comparing to our flux balance, (recall: $F = -\frac{dU}{dx}$)

$$\frac{dc}{dx} = \frac{Fc}{Df} = \frac{Fc}{k_B T} \Rightarrow \boxed{D = \frac{k_B T}{f}}$$

This is an example of a fluctuation-dissipation theorem, a profound result derived independently by Albert Einstein and William Sutherland in 1905.

Question: What is f?

Let's reason by dimensional considerations.

$$D = \left[\frac{L^2}{T}\right]$$

(the brackets mean "has units of")

$$k_B T = [\text{energy}] = \left[\frac{ML^2}{T^2}\right]$$

$$\Rightarrow f = \frac{k_B T}{D} = \left[\frac{M}{T}\right]$$

What might f depend on?

- Size of particle, $r = [L]$
- Viscosity of solvent, $\eta = \left[\frac{M}{LT}\right]$
- Density difference, particle − solvent, $\rho_{eff} = \left[\frac{M}{L^3}\right]$

To get something with units of M/T, we must use either ρ_{eff} or η. But there is nothing we can multiply ρ_{eff} by to get M/T. So, f does not depend on ρ_{eff}. Try η:

$$f \sim \eta r$$

For a sphere, the constant of proportionality is 6π. This was worked out by George Stokes: $f_{sphere} = 6\pi\eta r$.

Other shapes have varying factors, but always have the ηr proportionality.

So, for a diffusing sphere, we have the Stokes-Einstein-Sutherland relation:

$$\boxed{D = \frac{k_B T}{6\pi\eta r}}$$

Let's use this relation to guess the diffusion coefficients for biomolecules.

$k_B T \approx 4.1\ pN\text{-}nm$ (This is a great number to memorize: the thermal energy at physiological temp)

$r \approx 5\ nm$ (typical globular protein has diameter of $\approx 10\ nm$)

What is η? $\eta_{water} \approx 1\ cP = 10^{-3}\ \frac{N \cdot s}{m^2} = 10^{-9}\ \frac{pN \cdot s}{nm^2}$

So, in water, a typical globular protein has a diffsion coefficient of $D \approx \frac{4.1\ pN \cdot nm}{6\pi \cdot 10^{-9}\ pN \cdot s/nm^2 \cdot 5\ nm} \approx 4 \times 10^7\ \frac{nm^2}{s} = 40\ \frac{\mu m^2}{s}$.

Things diffuse much more slowly in cytoplasm because η is much higher. For example, D_{GFP} in *E. coli* is $\approx 6\ \frac{\mu m^2}{s}$, but in water, $D_{GFP} \approx 90\ \frac{\mu m^2}{s}$. (Konopka, et al., J. Bacteriol., 2006)

Note: The diffusion coefficient is also shape-dependent!

Question: How long does it take for a protein to diffuse across an *E. coli* cell?

$l_{E.coli} \approx 2\ \mu m$

$D \approx 6\ \frac{\mu m^2}{s}$

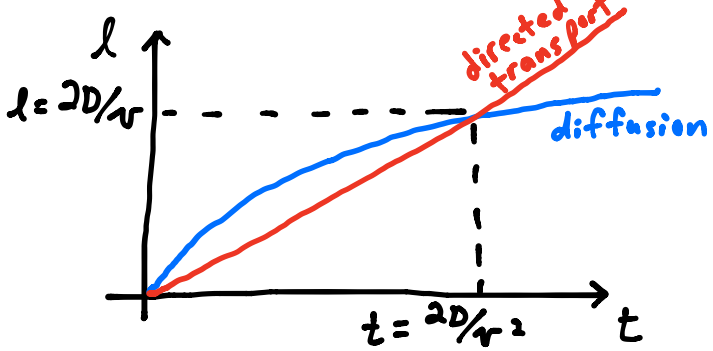
$\langle l_{E.coli}^2 \rangle = 2Dt \Rightarrow t = \frac{\langle l_{E.coli}^2 \rangle}{2D} \approx 0.3\ s.$

Conversely ask: how far will a protein diffuse in time t?

$l = \sqrt{\langle x^2 \rangle} = \sqrt{2Dt}$

By directed transport, typically done by motor proteins on filaments,

$l = vt$



$\Rightarrow$ diffusion faster for $l < \frac{2D}{v}$; directed transport faster for $l > \frac{2D}{v}$.

Typical motor protein speed $\simeq 300$ nm/s. So, for something like a globular protein, diffusion is faster for lengths less than

$$l_{crossover} \simeq \frac{2D}{v} \simeq \frac{2 \cdot 6\ \mu m^2/s}{0.3\ \mu m/s} \simeq 40\ \mu m.$$

It's interesting that bacteria have minimal directed transport compared to much larger eukaryotic cells.

Now: Other things that can be described as random walks

First: Active diffusion

The diffuion we just described is driven by thermal forces. It occurs at equilibrium.

Active diffusion is a non-equilibrium process in which energy is consumed. It is called active diffusion because the statistics are described by random walks.

Example: Bacterial motility by "run-and-tumble."

Run length = l_{step}

Bacterium changes direction after time τ.

Mean square displacement (1D): $\langle x^2 \rangle = \frac{l_{step}^2}{\tau} t$

$\Rightarrow D_{eff} = \frac{l_{step}^2}{2\tau}$. This is not thermal $\Longrightarrow D \neq \frac{k_B T}{f}$!

16 Analytical methods for biophysical chemistry

In this lecture, we will explore a few of the analytical techniques for measuring kinetic and equilibrium parameters of biochemical systems.

16.1 Equilibrium sedimentation

In equilibrium sedimentation experiments, a tube of solution of a protein or protein complex of interest is placed in a centrifuge. The concentration of protein is measured along the tube. The shape of this concentration profile is used to infer information about the molecular mass of the protein. This sounds like a silly way to compute the molecular mass of a biomolecule or biomolecular complex. Why not just do sequencing and add up the masses of the amino acids? This technique helps deduce which complexes form and in what stoichiometry, which is not really accessible from sequencing data.

Let ω be the angular velocity of the rotor of the centrifuge and r describe the distance from the center of the rotor to a given position in the tube of solution. Let ρ_{H_2O} be the density of the solvent and ρ_p be the density of the protein analyte of interest. Note that $\rho_p/\rho_{H_2O} \approx 1.4$ (BNID 104272). Let a be the radius of the analyte, which is what we seek to measure in this experiment.

Due to its density being greater than water, the protein will tend to fall toward the bottom of the tube with steady state velocity v. As it falls through the solvent, it experiences a friction f, such that it experiences a drag force of $F_{drag} = -fv$. As we have seen, for a spherical object, $f = 6\pi\eta a$, but we will use f for brevity for now. The analyte also experiences a centrifugal force, given by $F_{centrifugal} = m_e \omega^2 r$, where m_e is the effective mass of the protein. It is given by

$$m_e = M_a (1 - v_a \rho_{solvent}), \tag{16.1}$$

where $v_a = \partial V/\partial m_a$ is the change in volume of a solution when a mass of analyte is added, referred to as the partial specific volume of the analyte, and $\rho_{solvent}$ is the mass density of the solvent. The partial specific volume and mass density can be separately determined by measuring masses and volumes.

At steady state, the drag force balances the centrifugal force such that

$$F_{drag} + F_{centrifugal} = -fv + m_e \omega^2 r. \tag{16.2}$$

Solving for the sedimentation velocity, we have

$$v = \frac{m_e \omega^2 r}{f} = \frac{4\pi a^3}{3} \frac{(\rho_p - \rho_{H_2O})\omega^2 r}{f}. \tag{16.3}$$

We also know that at steady state, the total flux must vanish, meaning that

$$j_{\text{diffusive}} + j_{\text{sedimentation}} = -D\frac{\mathrm{d}c}{\mathrm{d}r} + vc = 0. \tag{16.4}$$

Therefore,

$$v = \frac{D}{c}\frac{\mathrm{d}c}{\mathrm{d}r} = D\frac{\mathrm{d}\ln c}{\mathrm{d}r}. \tag{16.5}$$

We now have two equations for the sedimentation velocity, which we can set equal to each other.

$$D\frac{\mathrm{d}\ln c}{\mathrm{d}r} = \frac{m_{\mathrm{e}}\,\omega^2\,r}{f}. \tag{16.6}$$

Integrating these equations over r gives

$$\int_{c_0}^{c}\mathrm{d}c'\,\frac{D}{c'} = D\ln\frac{c}{c_0} = \int_{r_0}^{r}\mathrm{d}r'\,\frac{m_{\mathrm{e}}\,\omega^2\,r}{f} = \frac{m_{\mathrm{e}}\,\omega^2(r^2-r_0^2)}{f}. \tag{16.7}$$

The Einstein-Smoluchowski relation gives that $Df = k_B T$, so we have

$$\ln\frac{c}{c_0} = \frac{m_{\mathrm{e}}\,\omega^2(r^2-r_0^2)}{2k_B T}. \tag{16.8}$$

We can measure all of the parameters in this equation except for m_{e}. So, with measured concentrations for each position r (including r_0), we can perform a regression to find m_e. From this, we can get the molecular mass via $M_{\mathrm{a}} = m_e/\left(1 - v_{\mathrm{a}}\,\rho_{\,\mathrm{solvent}}\right)$.

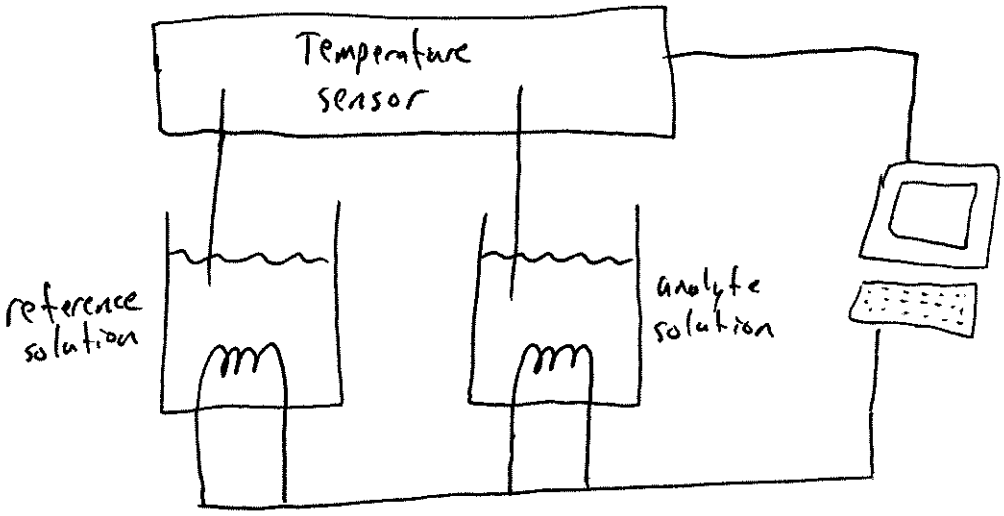
16.2 Differential scanning calorimetry

Calorimetric methods involve measuring heat input and output into a system of interest. Thermodynamic relations that we have derived thus far are then used to connect heat to properties of interest.

We first consider differential scanning calorimetry. In this

Please see scanned notes that follow.

Differential scanning calorimetry

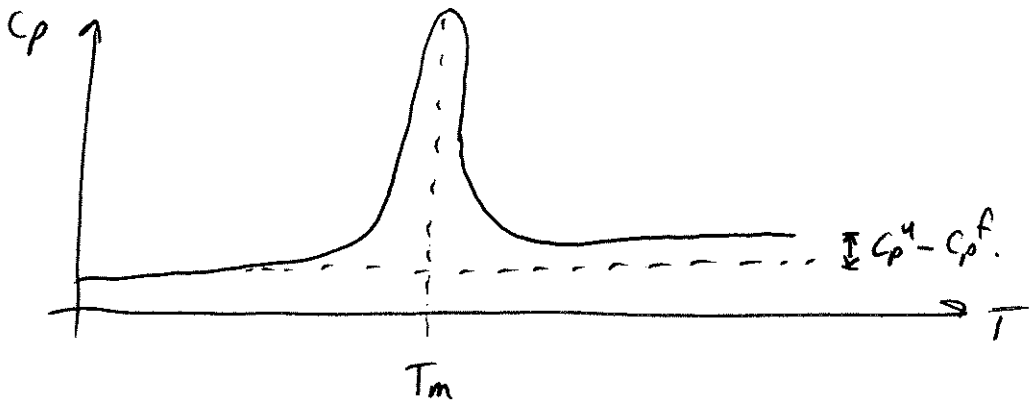


analyte is usually a protein.

Measure delivered heat to solutions and compute difference between the reference and analyte solution.

Recall that if we do things reversibly, $\left(\frac{\partial Q}{\partial T}\right)_{P,N} = T\left(\frac{\partial S}{\partial T}\right)_{P,N} = C_P$.

So, by measuring the heat input and temperature, we can measure C_P by differentiating. We do a temperature scan, and plot C_P vs T.



The C_p peaks at the melting temperature, T_m, since it takes extra heat, kind of like the heat of vaporization of water, to denature the analyte.

The difference in the baselines is the difference in heat capacities in the unfolded and folded states.

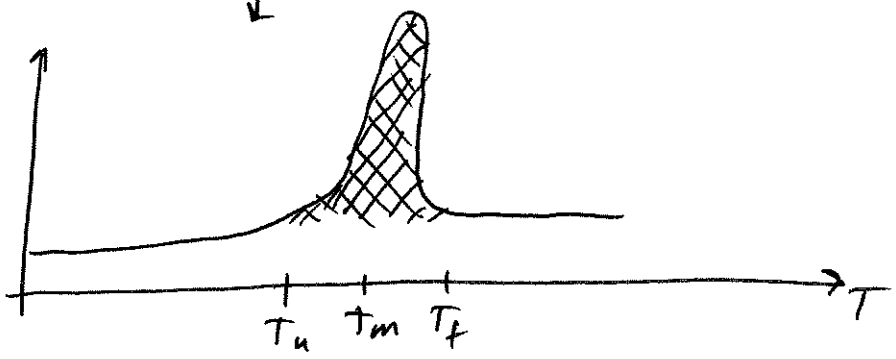
We can also work out the enthalpy change associated with unfolding.

Recall, $\left(\frac{\partial H}{\partial T}\right)_{P,N} = C_P$, or $\left(\frac{\partial h}{\partial T}\right)_{P,N} = C_p$ ← little C_p

$$\Rightarrow \int_{h_f}^{h_u} dh = \int_{T_f}^{T_u} dT\, C_p(T)$$

$$\Rightarrow h_u - h_f = \text{enthalpy of unfolding} = \int_{T_f}^{T_u} dT\, C_p(T)$$

area under curve.



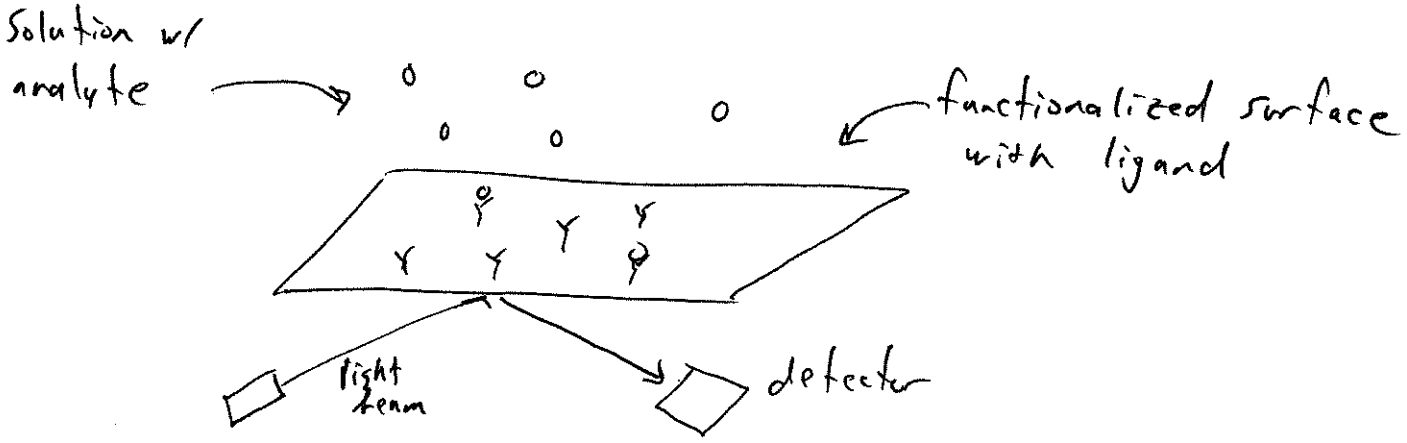
At T_m, the Gibbs free energy of folded & unfolded are equal, since the phases coexist at equilibrium. $\Rightarrow g_u - g_f = h_u - h_f - T_m(s_u - s_f) = 0$

$\Rightarrow s_u - s_f = \frac{h_u - h_f}{T_m}$, so we can also get the entropy change!

16.3 Surface plasmon resonance

Please see scanned notes that follow.

Surface Plasmon Resonance



Response is measured by deflection of surface.

Let R = response. This is a "read out function" of concentration of bound ligand. Let θ be the fraction of bound ligand. Let $R_0 = R(\theta=0)$ and $R_1 = R(\theta=1)$. Assuming R varies linearly with θ,

$$R = R_0 + (R_1 - R_0)\theta$$

Now, consider rxn $LA \underset{k_+}{\overset{k_-}{\rightleftharpoons}} L + A$. Let C_L^0 = total surface concentration of ligand.

Then, $$\frac{dC_{LA}}{dt} = k_+ C_L C_A - k_- C_{LA}$$

$$\Rightarrow \frac{d\, C_{LA}/C_L^0}{dt} = \frac{d\theta}{dt} = k_+ \frac{C_L}{C_L^0} C_A - k_- \frac{C_{LA}}{C_L^0} = k_+ C_A(1-\theta) - k_- \theta$$

or, more clearly, $$\frac{d\theta}{dt} = k_+ C_A(1-\theta) - k_- \theta = k_+ C_A - (k_- + k_+ C_A)\theta.$$

Note that C_A is held constant via flow of solution.

this is a first order linear differential eqtn., which can be solved by integrating factor to give:

$$\theta(t) = \frac{k_+ C_A}{k_- + k_+ C_A} + C' e^{-(k_- + k_+ C_A)\, t}$$ where C' is constant of integration.

Because the reaction is reversible, $\frac{k_-}{k_+} = K_d$ (where we report K_d in units of concentration)

So, $$\theta(t) = \frac{C_A/K_d}{1 + C_A/K_d} + C' e^{-(1 + C_A/K_d) k_- t}$$

If $\theta(t=0) = \theta_{init}$, then $C' = \theta_{init} - \frac{C_A/K_d}{1 + C_A/K_d}$, and

$$\theta(t) = \frac{C_A/K_d}{1 + C_A/K_d}\left(1 - e^{-(1 + C_A/K_d) k_- t}\right) + \theta_{init}\, e^{-(1 + C_A/K_d) k_- t}.$$

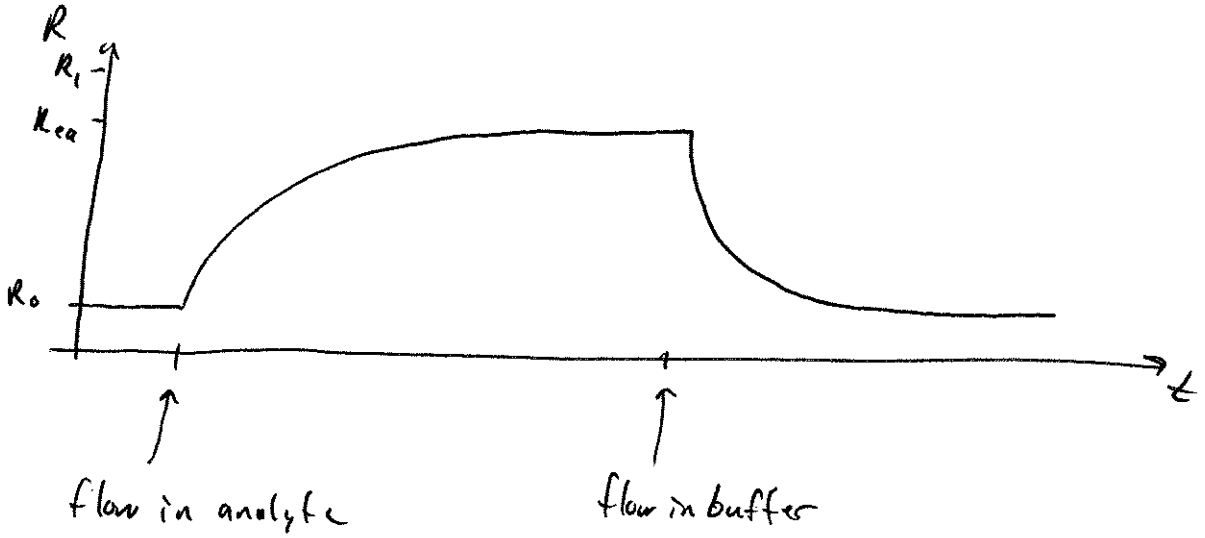
For ease of notation + to nondimensionalize, let

$$\tilde{t} = (1 + C_A/K_d) k_- t \quad \text{and} \quad \theta_{eq} = \frac{C_A/K_d}{1 + C_A/K_d}.$$

Then, $\theta(t) = \theta_{eq}\left(1 - e^{-\tilde{t}}\right) + \theta_{init}\, e^{-\tilde{t}}$.

In terms of R, $\theta = \frac{R - R_0}{R_1 - R_0}$, and we can measure R_0, which is R in the absence of analyte.

Experiment: Flow in analyte at conc. C_A. Observe response until saturated. Then flow in pure buffer. Observe response until depleted.



The decaying portion of the curve (to the right) has $C_A = 0$, so

$\theta(t) = \theta_{eq} e^{-\tilde{t}}$ where we define $\tilde{t} = 0$ to be when buffer is flowed in. (Here $\theta_{init} = \theta_{eq}$)

$= \theta_{eq} e^{-k_- t}$

or, $R(t) = R_0 + (R_{eq} - R_0) e^{-k_- t}$. We can get k_- by regression.

For the growth part, we have $\theta_{init} = 0$, so

$R(t) = (R_{eq} - R_0)(1 - e^{-(1 + C_A/K_d)k_- t})$, enabling us to set K_d, and therefore k_+ by regression.

Appendices

Appendix A: Algebraic manipulations in physical problems

Throughout this course, we often need to take complicated mathematical expressions and rearrange them into a more interpretable form. This is a crucial step in any theoretical analysis, as it allows us to gain insight from our mathematical expressions. To help keep things clear and simple, I find it is useful to follow the rules below.

- **Write what you know**: When you begin a model, write down expressions you know from your modeling assumptions or from physical laws.
- **Keep it simple**: As you are working through problems, keep expressions as simple as possible until they *need* to be complicated in order to make progress or gain insight. This means, for example, not to expand expressions into polynomials or insert expressions derived for given variables until it is necessary.
- **Dimensionless parameters**: When you identify dimensionless ratios of parameters, it is useful to redefine them as a single dimensionless parameter.
- **Dimensionless variables**: Similarly, when you identify a dimensionless ratio of a variable and a parameter, define a dimensionless variable.
- **Factor with unity**: Keeping in mind the keep-it-simple rule for *when* you do it, factor terms such that they have a the form $x = x_0 \times$ dimensionless factors. If a term consists of added dimensionless terms, factor into the form $(1 \pm$ dimensionless terms$)$ or $($dimensionless terms $- 1)$.
- **Easy limits**: When certain limits are of interest, consider the limits of simple expressions where possible. Expanding an expression as a Taylor series to linear order is often a good way to assess limiting expressions for small parameter values.

As an example of how we can go about deriving simple expressions for an analysis, let us consider a toy problem that draws on some thermodynamic principles of gases that we will not cover in this course. In the Fig. 8, is the schematic of a piston in a cylinder of length L with cross-sectional area A where the piston is connected to a spring with spring constant k. The cylinder and piston are *adiabatic*, meaning that no heat may be conducted through them; they are perfectly insulating. Initially, the pressure is equal on each side of the piston is p_0 and the spring is at its rest length (the length of the spring absent any applied force) of l. The temperature of the gas is T_0. Then, the right chamber is suddenly evacuated such that the gas in the left chamber expands, pushing the piston rightward against the spring. The gas has a per-particle heat capacity of c, which is independent of temperature and pressure. Our goal is to compute the equilibrium position x of the piston and the final temperature T and pressure p of the gas in the left chamber.

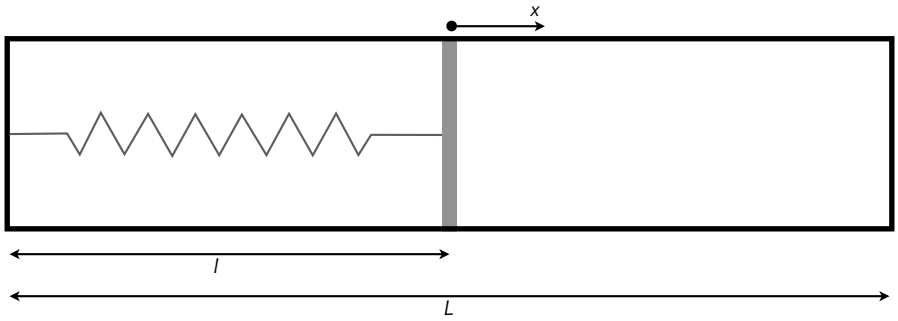


Figure 8: Side view of an adiabatic cylinder with a frictionless piston connected to a spring. The equilibrium length of the spring is l and the total length of the cylinder is L. It has a cross-sectional area of A.

According to the write-what-you-know rule, I will write down what I know. (Some of these principles are outside of the scope of the thermodynamics we cover in class, but the mathematical expressions are nonetheless simple.)

- I know from a balance of forces that at equilibrium, the pressure force is balanced by the restoring force of the spring. The former is pA and the latter is $-F_{\text{spring}} = kx$, giving

$$pA = kx. \tag{16.9}$$

- I also know that the ideal gas law should hold, such that

$$p = \frac{Nk_BT}{A(l+x)}. \tag{16.10}$$

since the volume of the left chamber is $A(l+x)$. Here, N is the number of gas particles in the left chamber.

> According to the factor-with-unity rule, it is useful to write sums as unity plus ratios, so we could write the expression for the pressure as
>
> $$p = \frac{Nk_BT}{Al}\frac{1}{1+x/l}.$$
>
> I have chosen not to now, since it adds clutter, and am sticking with the keep-it-simple rule. I am also not concerning myself with a more complicated expression for N right now, also in accordance with the keep-it-simple rule. We will get to both of these things later.

- The ideal gas law should also hold before evacuation of the right chamber, such that

$$p_0 = \frac{Nk_B T_0}{Al}. \tag{16.11}$$

- Another equation of state for an ideal gas relates the temperature to the internal energy. A spring is also present, which contributes $kx^2/2$ to the total internal energy, such that

$$E = NcT + kx^2/2. \tag{16.12}$$

- Because the cylinder and piston are well insulated, this is an adiabatic process, so the total internal energy does not change.

Now that we have what we know written out, we can devise a strategy to solve for the position of the equilibrium piston, x. The force balance, combined with the expression of for the pressure via the ideal gas law, give a relation between x and the equilibrium temperature T and the physical constants for this system. Next, we can get T as a function of x using the expression for the internal energy and noting the the internal energy does not change. Finally, we can get N as a function of the physical constants via (16.11). The expressions might get messy, but by looking at the simple expressions we already know and how they are connected, the path to a solution is clear.

Having the keep-it-simple rule in mind, we will not yet substitute the pressure in to the force balance, but just leave equations (16.9) and (16.10) for now. Instead, we will proceed to compute T in terms of x. Recalling another equation of state for an ideal gas, $E = NcT$, the internal energy in the left chamber prior to expansion is

$$E_0 = NcT_0. \tag{16.13}$$

The energy after expansion contains the thermal contributions from the gas, but also the energy in the spring, which is $kx^2/2$, giving

$$E = NcT + \frac{1}{2}kx^2. \tag{16.14}$$

Because the walls are adiabatic, the energy inside the chamber cannot change, so that $E = E_0$, giving

$$NcT_0 = NcT + \frac{1}{2}kx^2. \tag{16.15}$$

Rearranging, we get

$$T = T_0 - \frac{1}{2}\frac{kx^2}{Nc}. \tag{16.16}$$

Again, according to the factor-with-unity rule, I could write this as

$$T = T_0 \left(1 - \frac{1}{2}\frac{kx^2}{NcT_0}\right).$$

I am again choosing not to do this right now according to the keep-it-simple rule, though it would not add *that* much clutter if I did. We will regardless get to factoring-by-unity later in our work.

We are now ready to start combining things. We start with combining the force balance with our expression for the pressure via the ideal gas law.

$$pA = \frac{Nk_BT}{l+x} = kx. \tag{16.17}$$

Now, we will insert our expression for T.

$$\frac{Nk_B}{l+x}\left(T_0 - \frac{1}{2}\frac{kx^2}{Nc}\right) = kx. \tag{16.18}$$

We now have it; an equation involving x and the physical parameters of the system. Note that according to the keep-it-simple rule, we have not yet inserted our expression for N. To solve for x, we need to rearrange this equation to give us a quadratic equation.

$$k\left(1 + \frac{k_B}{2c}\right)x^2 + klx - Nk_BT_0 = 0. \tag{16.19}$$

A point where we start needing to work with polynomials is often a good place to start defining dimensionless parameters and dimensionless variables, as this can simplify the the expressions. We all have experience with polynomials leading to nasty expressions. We note that each term in the polynomial has dimension of energy, so we can divide by an energy scale, kl^2 so that all terms are dimensionless. We get

$$\left(1 + \frac{k_B}{2c}\right)\left(\frac{x}{l}\right)^2 + \frac{x}{l} - \frac{Nk_BT_0}{kl^2} = 0. \tag{16.20}$$

The equation is now a quadratic equation in x/l, which leads us to define a dimensionless variable $\tilde{x} = x/l$. This was a convenient time to apply the dimensionless-variable rule. We can also apply the dimensionless-parameter rule by noting we have two dimensionless parameters. The first is Nk_BT_0/kl^2, which we will define as ζ. If we write the ratio as

$$\zeta = \frac{Nk_BT_0}{kl^2} = \frac{Nk_BT_0/l^2}{k}, \tag{16.21}$$

we see that it is the ratio of a force-per-length due to the thermal energy of the gas (the numerator in the second expression) to the stiffness of the spring (the denominator). We could define our second dimensionless parameter as k_B/c, since that ratio appears, but it makes more physical sense to define our second dimensionless parameters as the inverse, c/k_B. This has the physical meaning of the heat capacity of the gas in units of k_B. Though outside the topics of this course, for a monatomic ideal gas, $c/k_B = 3/2$. Importantly, this tends to be not too far from unity for ideal gasses. So, we define a second dimensionless parameter

$$\tilde{c} = \frac{c}{k_B}. \tag{16.22}$$

We can now write the quadratic equation as

$$\left(1 + (2\tilde{c})^{-1}\right)\tilde{x}^2 + \tilde{x} - \zeta = 0. \tag{16.23}$$

Notice that this is a clean, tractable equation, even while employing the quadratic formula.

We can now solve for $\tilde{x}$ via the quadratic formula, choosing the root such that the piston moves rightward, to get

$$\begin{aligned}\tilde{x} &= \frac{-1 \pm \sqrt{1 + 4\zeta\,(1 + (2\tilde{c})^{-1})}}{2\,(1 + (2\tilde{c})^{-1})} \\ &= \frac{\tilde{c}}{1 + 2\tilde{c}}\left(\sqrt{1 + 4\zeta\,(1 + (2\tilde{c})^{-1})} - 1\right).\end{aligned} \tag{16.24}$$

Note that we have applied the factor-with-unity rule to get a more interpretable expression for $\tilde{x}$.

While this is a tidy expression, it still has N-dependence via ζ. To get an expression only in terms of p_0, T_0, and physical constants, we need an expression for N. We note that prior to evacuating the right chamber, we had

$$p_0 A l = N k_B T_0, \tag{16.25}$$

such that

$$N = \frac{p_0 A l}{k_B T_0}. \tag{16.26}$$

So, we can re-write

$$\zeta = \frac{N k_B T_0}{k l^2} = \frac{p_0 A / l}{k}, \tag{16.27}$$

which is a ratio of the force-per-length due to pressure exerted by the gas to the spring constant. Naturally, this has the same meaning as our previously defined ζ, since the pressure force of the gas is thermal in nature.

Finally, knowing x, we can write the temperature. We would like to express it in terms of our dimensionless variable and parameters. We did not do this immediately before because we had not yet decided on the dimensionless ratios we would use, but we can do it now. Starting with (16.16), we have

$$T = T_0 - \frac{1}{2}\frac{kx^2}{Nc} = T_0 - \frac{1}{2}\frac{kl^2\tilde{x}^2}{Nc}. \tag{16.28}$$

Inserting our expression for N, we get

$$T = T_0 - \frac{1}{2}\frac{kl^2\tilde{x}^2}{c}\frac{k_B T_0}{p_0 A l} = T_0\left(1 - \frac{\tilde{x}}{2\tilde{c}\zeta}\right), \tag{16.29}$$

where I have applied the factor-with-unity rule. The temperature decreases by the factor in parentheses from the starting temperature. I have chosen to leave this expression as

$$T = T_0\left(1 - \frac{\tilde{x}}{2\tilde{c}\zeta}\right), \tag{16.30}$$

because substituting the expression for $\tilde{x}$ will not add further insight. Finally, we can write the pressure from the force balance as

$$p = \frac{kl}{A}\tilde{x} = \frac{p_0}{\zeta}\tilde{x}. \tag{16.31}$$

So, in summary, we have

$$\frac{x}{l} = \frac{\tilde{c}}{1+2\tilde{c}}\left(\sqrt{1+4\zeta\,(1+(2\tilde{c})^{-1})} - 1\right), \tag{16.32}$$

$$\frac{T}{T_0} = 1 - \frac{x/l}{2\tilde{c}\zeta}, \tag{16.33}$$

$$\frac{p}{p_0} = \frac{1}{\zeta}\frac{x}{l}. \tag{16.34}$$

Note that we stipulate that $x \leq L$, lest the piston go through the end of the cylinder. So, if the above expression for x/l exceeds l/L, we set $x = L$.

Now let's consider limits. First, we consider the limit of a stiff spring. Note that "limit of a stiff spring" is not a precise statement, since we have to consider stiffness of the spring compared to another physical quantity. Fortunately, our dimensionless parameters allow us to do this! We can consider the limit where the energy stored in the spring is large compared to the thermal energy of the gas, such that $\zeta = Nk_BT_0/kl^2 \ll 1$. To take this limit, instead of expanding the entire expression

for $\tilde{x}$ in a Taylor series about $\zeta = 0$, we should use a simpler expression according to the easy limits rule. Instead, we compute the Taylor series of

$$\sqrt{1+ay} = 1 + \frac{ay}{2} + \mathcal{O}(y^2). \tag{16.35}$$

Using this easier-to-derive result, we can write, taking $a = 4\left(1+(2\tilde{c})^{-1}\right)$ for small ζ,

$$\sqrt{1+4\zeta\left(1+(2\tilde{c})^{-1}\right)} \approx 1 + 2\left(1+(2\tilde{c})^{-1}\right)\zeta, \tag{16.36}$$

such that

$$\tilde{x} = \frac{2\tilde{c}}{1+2\tilde{c}}\left(1+(2\tilde{c})^{-1}\right)\zeta = \zeta. \tag{16.37}$$

So, in the limit of a strong spring, we have

$$x = l\zeta, \tag{16.38}$$

$$T = T_0\left(1-(2\tilde{c})^{-1}\right), \tag{16.39}$$

$$p = p_0 \tag{16.40}$$

> You might think it even easier to consider the small ζ limit by going back to our quadratic equation (16.23). We have
>
> $$\left(1+(2\tilde{c})^{-1}\right)\tilde{x}^2 + \tilde{x} - \zeta = 0$$
>
> If we take $\zeta \approx 0$, we get
>
> $$\left(1+(2\tilde{c})^{-1}\right)\tilde{x}^2 + \tilde{x} = 0.$$
>
> This is now factorable and is easily solved. We get $\tilde{x} = 0$ and
>
> $$\tilde{x} = -\frac{1}{1+(2\tilde{c})^{-1}}.$$
>
> So, the root that gives the physical solution is identically zero. This is not right, as it includes only the zeroth order term in ζ, and not the first order term. In general, when working with polynomials and taking some coefficients to be small, you should use perturbation theory, which is beyond the scope of this class.

Now, let's consider the limit of a very weak spring, where ζ is large. We will also assume that L is sufficiently large that the piston does not hit the right wall of the

cylinder. In this case, the ζ term is dominant in the expression for $\tilde{x}$, giving

$$\tilde{x} \approx \frac{\tilde{c}}{1+2\tilde{c}}\sqrt{4\zeta\,(1+(2\tilde{c})^{-1})} = \sqrt{\frac{2\tilde{c}}{1+2\tilde{c}}\,\zeta}. \tag{16.41}$$

We can then compute the temperature and pressure in the limit of small ζ as

$$T = T_0\left(1-\frac{\tilde{x}}{2\tilde{c}\,\zeta}\right) \approx T_0\left(1-\frac{1}{\sqrt{2\tilde{c}(1+2\tilde{c})\,\zeta}}\right), \tag{16.42}$$

$$p = \frac{p_0}{\zeta}\,\frac{x}{l} \approx \sqrt{\frac{2\tilde{c}}{\zeta(1+2\tilde{c})}}. \tag{16.43}$$

It is important to note that in the weak spring limit, the displacement is roughly $\sqrt{\zeta}$ times the rest length of the spring. With ζ large, this is a very large displacement, and in practice Hooke's law would break down.

Because for ideal gases, $\tilde{c}$ tends to be close to order unity (roughly 3/2 for a monatomic ideal gas), the only dimensionless parameter we need to consider about is ζ, which is the ratio of the thermal energy of the gas in the chamber to the energy stored in the spring. We have done that, and we can plot the results, show in Fig. 9.

I hope that going through this exercise made clear the value of keeping it simple while going through algebraic manipulations. Application of the factor-with-unity, dimensionless-parameters, and dimensionless-variables rules greatly facility physical interpretation of the results you derive. Finally, limits are often useful to interpretation of results. For example, it is much easier to think about $x = l\zeta$ for small ζ that for the full expression for x that came from the quadratic formula.

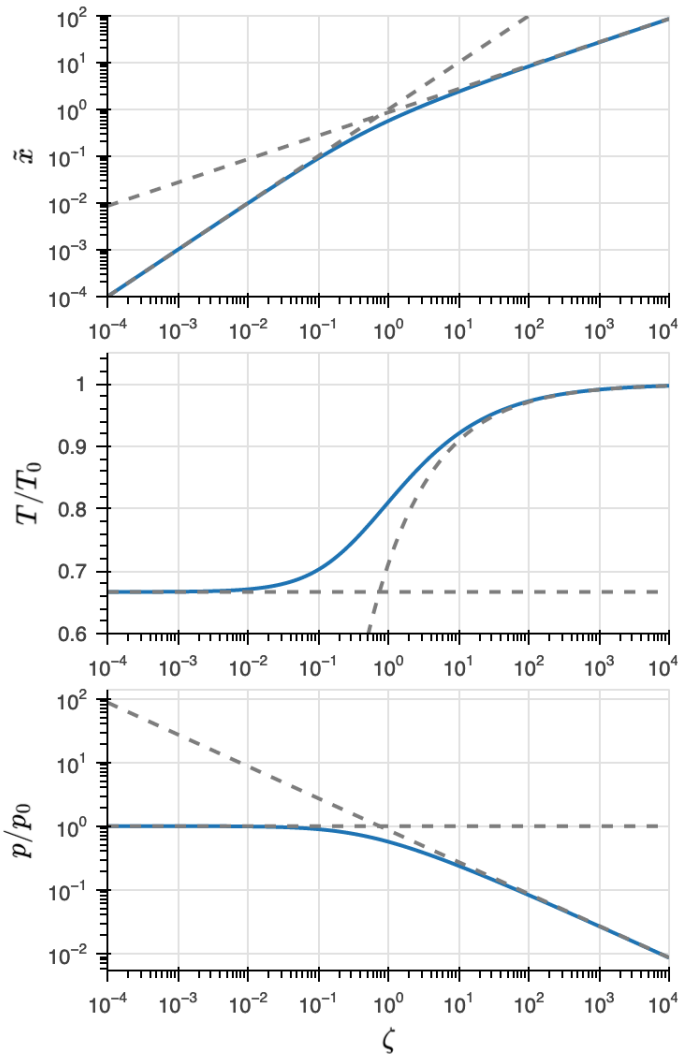


Figure 9: Dimensionless piston position, temperature, and pressure as a function of ζ. The dashed lines are the low- and high-ζ limits. The code to generate these plots are in Listing 2.

Appendix B: Code listings

```
import numpy as np
import scipy.integrate

import bokeh.io
import bokeh.plotting


def semibatch_rhs(N, t, kappa):
    """Right-hand side for semibatch reactor dynamics for
    A + B -> C, with feed of A."""
```

```
    NA, NB = N
    dNA_dt = -kappa * NA * NB / (1 + t)
    dNB_dt = 1 - dNA_dt

    return np.array([dNA_dt, dNB_dt])


# Parameters
t_end = 5
t = np.linspace(0, t_end, 400)
N0 = np.array([10, 0])
kappa_vals = [0.3, 1, 3]

# Solve and build plots
figs = []
for i, kappa in enumerate(kappa_vals):
    # Solve
    N = scipy.integrate.odeint(semibatch_rhs, N0, t, args=(kappa,))

    # Compute number of AB molecules
    NAB = N0[0] - N[:, 0]

    # Compute volume to get dimensionless concentrations
    V = 1 + t

    # Build plots
    p = bokeh.plotting.figure(
        frame_width=400,
        frame_height=100,
        x_axis_label="tτ/" if i == 2 else "",
        y_axis_label="nondim. conc.",
        x_range=[0, 5],
    )
    p.line(t, N[:, 0] / V, line_width=2, legend_label="A")
    p.line(
        t,
        N[:, 1] / V,
        line_color=bokeh.palettes.Category10_3[1],
        line_width=2,
        legend_label="B",
    )
    p.line(
        t,
        NAB / V,
        line_color=bokeh.palettes.Category10_3[2],
        line_width=2,
        legend_label="AB",
    )
    p.title = f"κ = {kappa}"
    if i > 0:
        p.legend.visible = False
    figs.append(p)
```

```
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64
65 bokeh.io.show(bokeh.layouts.gridplot(figs, ncols=1))
```

Listing 1: Semibatch reactor time course

```
import numpy as np

import bokeh.io
import bokeh.plotting

# Parameters (c for monatomic ideal gas)
c = 1.5
zeta = np.logspace(-4, 4, 200)

# Full analytical solutions
x = c / (1 + 2 * c) * (np.sqrt(1 + 4 * zeta * (1 + 1 / 2 / c)) - 1)
T = 1 - x / 2 / c / zeta
p = x / zeta

# Approximate solutions for low zeta
x_low = zeta
T_low = 1 - 1 / 2 / c
p_low = 1

# Approximate solutions for high zeta
x_high = np.sqrt(2 * c * zeta / (1 + 2 * c))
T_high = 1 - 1 / np.sqrt(2 * c * (1 + 2 * c) * zeta)
p_high = np.sqrt(2 * c / zeta / (1 + 2 * c))

# Set up plots
plot_kwargs = dict(
    frame_width=300,
    frame_height=150,
    x_axis_type="log",
    x_range=[1e-4, 1e4],
    align="end",
    toolbar_location=None,
)
plot_x = bokeh.plotting.figure(
    y_axis_label="$$\\tilde{x}$$",
    y_axis_type="log",
    y_range=[1e-4, 1e2],
    **plot_kwargs,
)

plot_T = bokeh.plotting.figure(
    y_axis_label="$$T/T_0$$",
    y_range=[0.6, 1.05],
    **plot_kwargs,
)

plot_p = bokeh.plotting.figure(
    x_axis_label="$$\\zeta$$",
    y_axis_label="$$p/p_0$$",
    y_axis_type="log",
    **plot_kwargs,
```

```
)

# Populate glyphs
limit_kwargs = dict(line_width=2, line_color="gray", line_dash="
    dashed")
plot_x.line(zeta, x, line_width=2)
plot_x.line(zeta, x_low, **limit_kwargs)
plot_x.line(zeta, x_high, **limit_kwargs)

plot_T.line(zeta, T, line_width=2)
plot_T.line(zeta, T_low, **limit_kwargs)
plot_T.line(zeta, T_high, **limit_kwargs)

plot_p.line(zeta, p, line_width=2)
plot_p.line(zeta, p_low, **limit_kwargs)
plot_p.line(zeta, p_high, **limit_kwargs)

# Display
bokeh.io.show(bokeh.layouts.column([plot_x, plot_T, plot_p]))
```

Listing 2: Piston-spring analysis