Topic 3: Probability Theory and Boltzmann Distribution
The Boltzmann Distribution:

So far we’ve been talking about Ideal gases, but what about real systems

Consider a small system of particles that finds itself in a large thermal reservoir, that is at fixed temperature

We want to calculate statistical properties of this system

Want to find the probability of finding the system in each of its possible states

We will derive the Boltzmann distribution
Microstates:

What we mean, is that the small system can be in a variety of possible configurations, each with its own associated energy $E$.

Given that this system is in contact with the thermal reservoir, what is the likelihood of observing a particular configuration?

We call these configurations of the small system, microstates.

need to be able to count these microstates

Figure 6.2 Physical Biology of the Cell, 2nd. (© Garland Science 2013)
Both systems, A and B are at temperature $T$

Total energy is constant: $E_{tot} = E_A + E_B$

Equilibrium: every state in the combined system has energy $E_{tot}$ and so has the same probability of occurring

Using this postulate, what does it imply for $P(E_A)$?

For A, in a state with energy $E_A$, how many states in system B are there? (the one that has the most states in B will be the most likely to be observed)

We know the entropy in B:

$$S(E_B) = k_B \log(\Omega(E_B))$$

thus the number of states in B is $\Omega(E_B) = \exp\left(\frac{S(E_B)}{k_B}\right)$
Boltzmann distribution derivation

But we know that $E_B = E_{tot} - E_A$, so it depends on the energy in $A$, so rewrite

$$\Omega(E_{tot} - E_A) = \exp(S_B(E_{tot} - E_A))$$

Thus the # of states in $B$ depends on what state $A$ is in.

So prob of $A$ being in $E_A$ is \( P_0 \cdot \text{(# of B states)} \)

or \( P(E_A) \propto P_0 \exp(E_{tot} - E_A) = P_0 e^{S_B(E_{tot} - E_A)/k_B} \)

Since $E_A \ll E_{tot}$, we can expand $S_B(E_{tot} - E_A)$:

$$S_B(E_{tot} - E_A) \approx S_B(E_{tot}) - E_A \frac{dS_B}{dE_B}$$

so

$$P(E_A) \propto \left( P_0 e^{S_B(E_{tot})/k_B} \right) e^{-E_A/k_B T_B}$$

or

$$P(E_A) = A e^{-E_A/k_B T}$$
$P(E_\alpha) = A \exp\left(-\frac{E_\alpha}{k_BT}\right)$

Find $A$ by normalizing. $\sum_{E_\alpha} P(E_\alpha) = 1$

$\Rightarrow A = \left(\sum_{E_\alpha} \exp\left(-\frac{E_\alpha}{k_BT}\right)\right)^{-1}$

or

$A = \frac{1}{Z}$ where $Z = \sum_{E_\alpha} \exp\left(-\frac{E_\alpha}{k_BT}\right)$

$Z \equiv$ Partition function.

$P(E_\alpha) = \frac{1}{Z} \exp\left(-\frac{E_\alpha}{k_BT}\right) \equiv$ Boltzmann distribution

commit this equation to memory – you will use it lots

Thus for a system at temperature $T$, the probability of a state with energy, $E$ is given by the above distribution. The rest is just application of this equation.
Two-state systems: Suppose \( \Delta \) can exist in 2 states

\[
\Delta E \\
\Delta E
\]

\[ P_1, P_2 = \frac{e^{\frac{-E_1/k_B T}{}}}{e^{\frac{-E_1+\Delta E/k_B T}{}}}
\]

\[ P_1 + P_2 = 1 \]

Combining \( 1 \) & \( 2 \) gives:

\[
P_1 = \frac{1}{1 + e^{\frac{-\Delta E/k_B T}{}}}; \quad P_2 = \frac{1}{1 + e^{\frac{\Delta E/k_B T}{}}}
\]

\[ P_1 \]

\[ P_2 \]

@ low \( T \), system is in lowest energy state, \( E \),

as \( T \) rises, both states become equally likely.
Two state system: an ion channel

\[ t_C = \exp\left(-\frac{\varepsilon_C}{kT}\right) / \exp(-\frac{\varepsilon_O}{kT}) \]
Boltzmann and chemical kinetics:

Chemical equilibrium occurs when the flow from state 1 to state 2 is the same as the flow from state 2 to state 1. What does this imply?

\[ S_2 \xrightarrow{k_+} S_1 \]

\[ k_+ = \frac{\text{prob of going from } S_2 \text{ to } S_1 (\text{chance of going over barrier})}{\text{unit time}} \]

So, \[ k_+ = Ce^{-E_b/kT} \] — chance of having an energy path would put you over the barrier.

So, the number of \( N_2 \) molecules going to \( S_1 \) is

\[ N_2 k_+ = N_1 Ce^{-E_b/kT} \]
Chemical equilibrium:

What about \( S_1 \rightarrow S_2 \)? To go from \( S_1 \rightarrow S_2 \) requires going over a barrier of \( \Delta E = \Delta E_{1\rightarrow2} + E_B \)

so

\[
 k^- = c e^{-\left(\Delta E_{1\rightarrow2} + E_B\right)/kT}
\]

- # of \( N_1 \) molecules making transition \( S_1 \rightarrow S_2 \)

\[
 N_1 k^- = N_1 c e^{-\left(\Delta E + E_B\right)/kT}
\]

- Equilibrium:

\[
 N_2 k^+ = N_1 k^- \\
 or \\
 \frac{N_2}{N_1} \frac{c}{c} = e^{-\Delta E_{1\rightarrow2}/kT} = \frac{P_2}{P_1}
\]

So at equilibrium the distribution of particles only depends on the energy difference between the states.

What happened to the energy barrier? It serves to set how long it takes to reach equilibrium. Larger the barrier, the longer it will take to reach equilibrium.
Dwell times:

We’ve just found the likelihood of being in each state, but on average how long will you spend in each state? This is known as the dwell time.

What are the distribution of dwell times?

\[ P_{2\rightarrow 1}(t) \equiv \text{the probability that you stay in state 2 for a time, } t \]

\[
\text{Start: 0@ time t there are } N(t) \text{ molecules in } S_2 \\
\text{\quad 0 after a time } dt, \quad -k_+dt N(t) \text{ will have left} \\
\text{so} \\
N(t+dt) = (1 - k_+dt)N(t) \quad \text{(with } N(0) = N_0) \\
\text{So we:} \\
N(t) = N_0 e^{-k_+t}
\]

\[
\int P_{2\rightarrow 1}(t) dt = (\text{prob of surviving in } S_2 \text{ till } t)(\text{prob of jumping in } dt)
\]

or

\[
P_{2\rightarrow 1}(t) dt = \left( \frac{N(t)}{N_0} \right) (k_+ dt)
\]

so

\[
P_{2\rightarrow 1}(t) = k_+ e^{-k_+t}
\]

exponential distribution
Dwell times:

Many reactions occur quickly, but some are very slow.

The energy barrier affects the rate. From Boltzmann,

\[ \text{rate (2} \to \text{1)} = (\text{attempt rate}) \times (\text{probability of jumping barrier from 1} \to \text{2}) \]

or

\[ k_+ = \nu \exp\left(-\frac{E_B}{k_B T}\right) \]

so larger barrier \(\to\) slower rate and longer dwell times.

So dwell times are distributed exponentially.

\[ p_{2\to1}(t) = k_+ e^{-k_+ t} \]
Generalizing to systems with degeneracies

Previously, we assumed that the small system had states with energy $E$, and these states had no degeneracy (i.e. there is only one configuration associated with each energy $E$).

But what if there are multiple configurations that have the same energy $E$?

<table>
<thead>
<tr>
<th>$E_1$</th>
<th>$E_2$</th>
<th>$E_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td># of states, $\Omega_1$</td>
<td># of states, $\Omega_2$</td>
<td># of states, $\Omega_3$</td>
</tr>
</tbody>
</table>

Use Boltzmann to calculate, probability of state 1, etc.

$$P_1 = \frac{\Omega_1 \exp\left(-\frac{E_1}{kT}\right)}{\Omega_1 \exp\left(-\frac{E_1}{kT}\right) + \Omega_2 \exp\left(-\frac{E_2}{kT}\right) + \Omega_3 \exp\left(-\frac{E_3}{kT}\right)} = \exp\left[-\frac{1}{kT}(E_1 - kT \log \Omega_1)\right]$$

so

$$P_i = \frac{1}{Z} \exp\left(-\frac{G_i}{kT}\right) \text{ where } Z = \sum_i \exp\left(-\frac{G_i}{kT}\right) \text{ and } G_i = E_i - TS_i$$
Application to RNA folding

Using an optical tweezer, the Bustamante lab looked at how a single RNA molecule unfolded under different applied forces.

Measured the probability of being folded

Measured dwell times
The optical trap does work when the RNA unfolds a distance, $\Delta z$. So the free energy difference between the unfolded and folded in the presence of a force is:

$$\Delta G = G_I - G_{II} - f \Delta z = \Delta G_0 - f \Delta z$$

So the probability of being folded is:

$$P_{II} = \frac{\exp \left( - \frac{G_{II}}{kT} \right)}{\exp \left( - \frac{G_{II}}{kT} \right) + \exp \left( - \frac{G_I - f \Delta z}{kT} \right)} = \frac{1}{1 + \exp \left( - \frac{\Delta G_0 - f \Delta z}{kT} \right)}$$
RNA folding dwell times:

- Unfolded
- Folded

Record the duration of time spent in a given state.

Histogram these values.
RNA folding dwell times:

- folded
- unfolded

Increasing force:

Decreasing force:

Energy barrier:

Recall, dwell time probability is:

\[ p_{2 \rightarrow 1}(t) = k_+ e^{-k_+ t} \]

where,

\[ k_+ = v \exp\left(- \frac{E_B - f \Delta z}{k_B T}\right) \]

Applying the force lowers the barrier between the folded and unfolded state.

So in the optical tweezers experiment, they can measure the distribution and see the effect of force.
The Boltzmann distribution gives us the ability to calculate the probability of observing a system at finite temperature in any particular microstate.

This probability only depends on the energy (free energy) of the state.

Energy barriers affect the kinetics of reaction, not the final equilibrium.

Dwell times in a particular state depend on the barriers between the states.

Optical tweezer experiments can measure directly the equilibrium likelihood and dwell times of each state.