BE/APh 161: Physical Biology of the Cell

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5 Two-state models case study: mechanosensitive ion channels

In the last lecture, we worked through some basic ideas of statistical mechanics and applied them to ligand-receptor binding. The simple ligand-receptor binding example belongs to a class of **two-state models**. As the name suggests, these are models where there are two states to consider. In the ligand-receptor binding example, there were two states for the receptor, bound and unbound. A great many systems may be modeled with two-state models, and we can use the tools of statistical mechanics to derive useful expressions describing their equilibrium behavior.

In this lecture, we will investigate another two-state model, this time ion channels. **Ion channels** are transmembrane protein complexes that can open and close to mediate the transport of ions in and out of a cell. We will use mechanosensitve ion channels, such as Mscl in *E. coli* as our first case study in two-state models.

5.1 Experimental analysis of ion channels

Bert Sakmann and Erwin Neher developed the **patch clamp technique** whereby researchers can measure current through a single ion channel. Such readings can give traces like those shown in Fig. 9.

If we consider a long time trace, we can compute p_open, the equilibrium probability that an ion channel is open, as the total time during the trace where the channel is open divided by the total time of the trace. The greater p_open is, the more ions can flow through it per unit time.

5.2 A simple two-state model for an ion channel

In order to compute p_open for an ion channel, we define two states, open and closed. We can assign energies to these two states, E_open and E_closed. We can then write a states and weights table, as in the previous lecture.

state	**energy**	**statistical weight**
closed	E_closed	$\mathrm{e}^{-\beta E_\text{closed}}$
open	E_open	$\mathrm{e}^{-\beta E_\text{open}}$

We can then compute the probability that the channel is open as

$$p_\text{open} = \frac{\mathrm{e}^{-\beta E_\text{open}}}{\mathrm{e}^{-\beta E_\text{open}} + \mathrm{e}^{-\beta E_\text{closed}}} = \frac{\mathrm{e}^{-\beta (E_\text{open} - E_\text{closed})}}{1 + \mathrm{e}^{-\beta (E_\text{open} - E_\text{closed})}}. \tag{5.1}$$

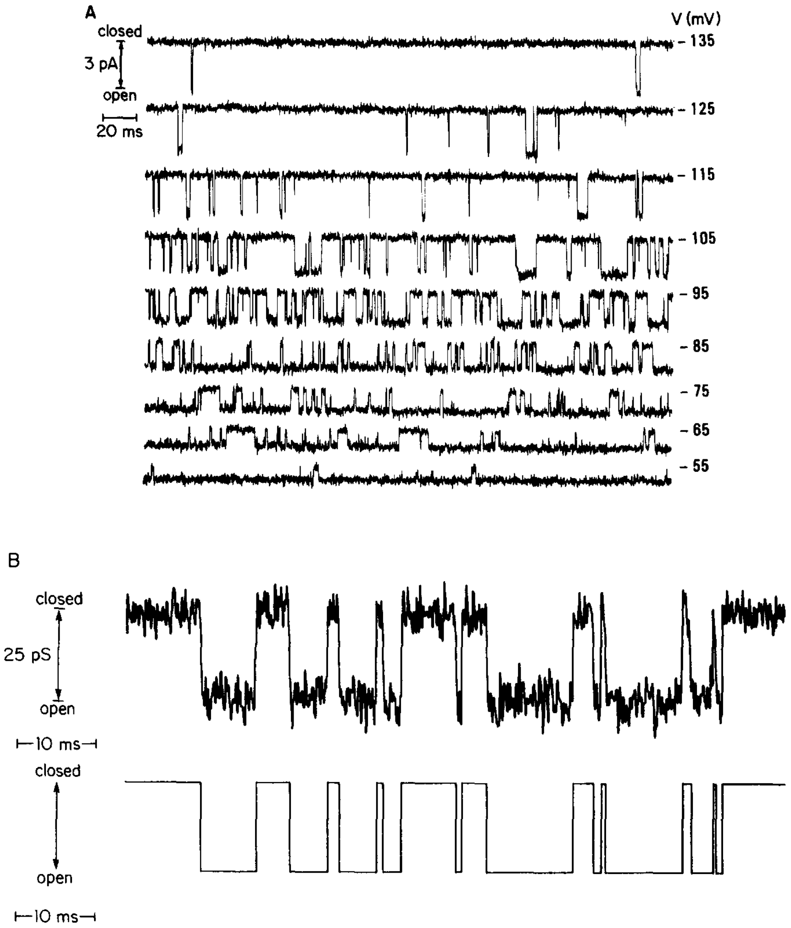

Figure 9: Patch clamp recordings of a single sodium ion channel in a reconstituted lipid bilayer. A. Recordings of current taken at different voltages. For a voltage of high magnitude, the channel has a constant current, indicating it is almost always open. For voltage of low magnitude, it is closed. B. Detail of the trace at -95 mV. The bottom trace shows a digitized version, displaying when the channel is open or closed. Figure taken from Keller, et al., *J. Gen. Physiol.*, **88**, 1–13, 1986..

Naturally, the open and closed energies will depend on the voltage, which will give p_{open} as a function of voltage. This is an example of a **voltage gaged ion channel**. But for our present case study, we will consider **mechanosensitive ion channels**, where p_{open} (via the energy of the two states of the channel) depends on the *tension* in the membrane. So, our goal is to write

$$E_{\text{open}} = E_{\text{open}}(\gamma), \tag{5.2}$$

where γ is the membrane tension, and then compute p_{open} using the Boltzmann weights.

Before we proceed to this calculation, we first provide some context as to why a cell would need mechanosensitive ion channels to deal with sudden changes in pressure due to osmotic shock.

5.3 Osmotic pressure

Osmotic pressure, is a pressure exerted across a membrane due to differences in concentration of solute on either side of the membrane. In the case of Mscl, the solute is positive ions. We can understand osmotic pressure by looking at the thermodynamics of dilute solutions. The chemical potential of water on either side of a cell membrane, must be equal at equilibrium. That is, the chemical potential of water in the cell must equal that in the environment.

$$\mu^{\text{cell}}_{\text{H}_2\text{O}} = \mu^{\text{env}}_{\text{H}_2\text{O}}. \tag{5.3}$$

In the previous lecture in equation (4.56), I stated without proof (see section 6.2.2 of *PBoC2*) that the chemical potential of water in a dilute solution is

$$\mu_{\text{H}_2\text{O}}(p, T) = \mu^0_{\text{H}_2\text{O}}(p, T) - k_BTx, \tag{5.4}$$

where x is the mole fraction of solute molecules. Note that the chemical potential is in general a function of pressure and temperature. So, at equilibrium, we have

$$\mu^0_{\text{H}_2\text{O}}(p_{\text{cell}}, T) - k_BTx_{\text{cell}} = \mu^0_{\text{H}_2\text{O}}(p_{\text{env}}, T) - k_BTx_{\text{env}}. \tag{5.5}$$

This implies that

$$\mu^0_{\text{H}_2\text{O}}(p_{\text{cell}}, T) - \mu^0_{\text{H}_2\text{O}}(p_{\text{env}}, T) = k_BT(x_{\text{cell}} - x_{\text{env}}). \tag{5.6}$$

Note that we have assumed thermal equilibrium. Then, if the concentration of solute molecules in the cell is different than in the environment, $x_{\text{cell}} \neq x_{\text{env}}$, then the inside and outside of the cell must have different pressure. This difference in pressure, $\Pi \equiv p_{\text{cell}} - p_{\text{env}}$, is called the osmotic pressure. To proceed, we can expand the left hand side of the above equation about $\Pi = p_{\text{cell}} - p_{\text{env}} = 0$ to first order to get

$$\mu^0_{\text{H}_2\text{O}}(p_{\text{cell}}, T) - \mu^0_{\text{H}_2\text{O}}(p_{\text{env}}, T) \approx \left(\frac{\partial \mu^0_{\text{H}_2\text{O}}}{\partial p}\right) \Pi. \tag{5.7}$$

The differential in this equation is the volume of a water molecule, as we know from thermodynamics.[2]

$$\frac{\partial \mu^0_{\text{H}_2\text{O}}}{\partial p} = v_{\text{H}_2\text{O}} = V/N_{\text{H}_2\text{O}}. \tag{5.8}$$

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[2] To see this, consider the total Legendre transform of the free energy, $0 = -S\,\mathrm{d}T + V\,\mathrm{d}p - N\,\mathrm{d}\mu$, and compute $(\partial\mu/\partial p)_T$.

Thus, we have

$$V/N_{\mathrm{H_2O}}\,\Pi = k_BT(x_{\mathrm{cell}} - x_{\mathrm{env}}). \tag{5.9}$$

Recall that $x_{\mathrm{cell}} \approx N^{\mathrm{cell}}_{\mathrm{solute}}/N_{\mathrm{H_2O}}$. Using this fact, we have

$$\Pi = k_BT(c_{\mathrm{cell}} - c_{\mathrm{env}}), \tag{5.10}$$

were c represents a concentration, N_{solute}/V.

The typical concentration of positive ions in *E. coli* is approximately 200 mM (BNID 104049), or about 0.1 molecules per cubic nanometer. Thus, the osmotic pressure in an *E. coli* cell, assuming that $c_{\mathrm{env}} \approx 0$ (which would be the case if you put a cell in deionized water) is

$$\Pi \approx 4\,\text{pN-nm} \times 0.1\,\text{nm}^{-3} = 0.4\,\text{pN/nm}^2. \tag{5.11}$$

Given the conversion that 1 pN/nm^2 $\approx$ 10 atm, the osmotic pressure in *E. coli* in deionized water is approximately 4 atm. The cell can handle the pressure with its cell wall, but you can imagine that if you rapidly changed the ionic conditions outside the cell, it suddenly has to withstand a very large pressure, which can lead to the cell bursting. Mechanosensitive ion channels respond to increased membrane tension as a result of osmotic shock to let ions in or out to relieve osmotic pressure.

5.4 Tension and the ion channel

When the ion channel is closed, the membrane is more stretched than when it is open. This is because a closed channel pulls the membrane more taught, and an open membrane can relieve the tension. The opening of the channel leads to a change in total area of the surface of the cell, ΔA. We should take into account the areal stretch of the membrane when considering the energetics of channel opening. So, we have $E^{\mathrm{stretch}} = E_{\mathrm{stretch}}(\Delta A)$, and define $\Delta A = 0$ for the closed state. We write E^{stretch} as a Taylor series in ΔA about $\Delta A = 0$. To first order,

$$E^{\mathrm{stretch}}_{\mathrm{open}} = E^{\mathrm{stretch}}_{\mathrm{closed}} - \gamma\,\Delta A. \tag{5.12}$$

It is clear from the Taylor expansion that γ is a tension (with dimension force per length). We have chosen a negative sign to ensure that γ is positive under our definition that ΔA is positive. The stretching energy of the open state is less than the closed state. Thus, we have

$$E_{\mathrm{closed}} = E^0_{\mathrm{closed}} + E^{\mathrm{stretch}}_{\mathrm{closed}}, \tag{5.13}$$

$$E_{\mathrm{open}} = E^0_{\mathrm{open}} + E^{\mathrm{stretch}}_{\mathrm{closed}} - \gamma\,\Delta A. \tag{5.14}$$

We have divided the energy of a state into the energetics associated with the state of the channel itself, marked by a naught superscript, and the energy associated with stretching the membrane. If we define $E^0_{\mathrm{closed}} + E^{\mathrm{stretch}}_{\mathrm{closed}}$ as our reference energy, and $\varepsilon \equiv E^0_{\mathrm{open}} - E^0_{\mathrm{closed}}$, our updated states and weights table is as follows.

state	**energy**	**statistical weight**
closed	0	1
open	$\varepsilon - \gamma\,\Delta A$	$\mathrm{e}^{-\beta(\varepsilon-\gamma\,\Delta A)}$

We can now write our updated expression for the probability of the ion channel being open as a function of the membrane tension γ.

$$p_{\text{open}} = \frac{\mathrm{e}^{-\beta(\varepsilon-\gamma\,\Delta A)}}{1+\mathrm{e}^{-\beta(\varepsilon-\gamma\,\Delta A)}}. \tag{5.15}$$

5.5 Determining the parameters

As we have seen again and again in the course, physical modeling of cellular systems exposes measurable parameters and testable hypotheses. So, can we do a patch clamp experiment to determine the parameters? Perozo and coworkers (Perozo, et al., *Nat. Struct. Biol.*, **9**, 696–703, 2002) did just that. They adjusted the applied pressure across a reconstituted membrane and could measure the current through a single Mscl channel. They then computed p_{open} as I described above for the voltage gated ion channel. I digitized the data from their measurements and show them in Fig. 10.

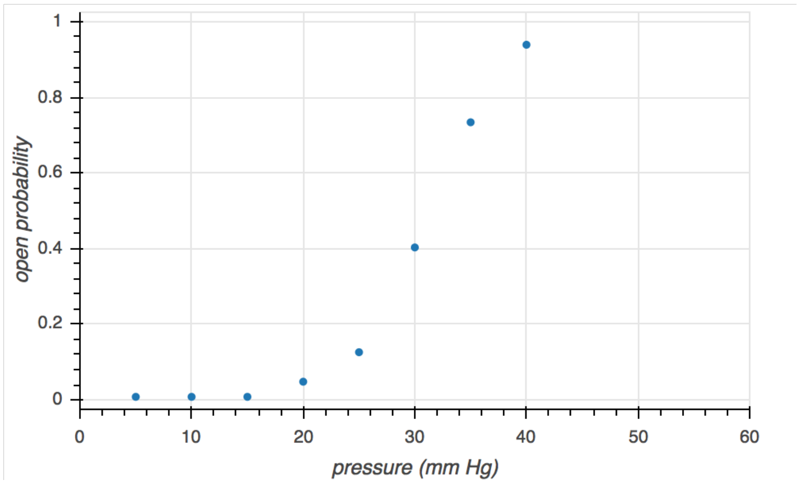

Figure 10: Digitized data from a patch clamp experiment from Perozo, et al., *Nat. Struct. Biol.*, **9**, 696–703, 2002.

As we will derive when we do membrane mechanics later in the course, the tension γ in the membrane is directly proportional to the applied pressure. Defining the constant of proportionality to be α, we can write the theoretical curve describing the experimental data as

$$p_{\text{open}} = \frac{\mathrm{e}^{-\beta(\varepsilon - \alpha p \Delta A)}}{1 + \mathrm{e}^{-\beta(\varepsilon - \alpha p \Delta A)}}, \tag{5.16}$$

where p is the applied pressure. This expression has five parameters, β, α, ΔA, E^0_{open}, and E^0_{closed}, where the last two are present in ε. As we can already see, the equation we have derived for p_{open} can only delineate the *difference* in the open and closed energies of the ion channel, parametrized by α. The experiments were done at room temperature (about 295 K), so we know $\beta \approx 1/(4 \text{ pN-nm})$. We might know what ΔA is from structural studies, but let's assume we do not know it. By defining $a = \alpha\, \Delta A$, and re-writing p_{open},

$$p_{\text{open}} = \frac{\mathrm{e}^{-\beta\,\varepsilon}\mathrm{e}^{\beta a p}}{1 + \mathrm{e}^{-\beta\,\varepsilon}\mathrm{e}^{\beta a p}}, \tag{5.17}$$

we see that we can only determine two constants from measurements of p_{open} (given that we know β), ε, the difference in energy of the open and closed states of the channel in the absence of tension, and a, which describes how tension on the membrane serves to open channels.

We can perform a nonlinear regression to obtain estimates for the parameters $\beta\,\varepsilon$ and βa. The code to do the regression appears below (with some LaTeX-based problems with displaying unicode at the very end of the script). To do the regression, we use least squares, as implement in SciPy.

Performing the regression, we get that the most probable parameter values are $\beta\,\varepsilon = 9.2$ and $\beta a = 0.3$ (mm Hg)$^{-1}$. The result is shown in Fig. 11.

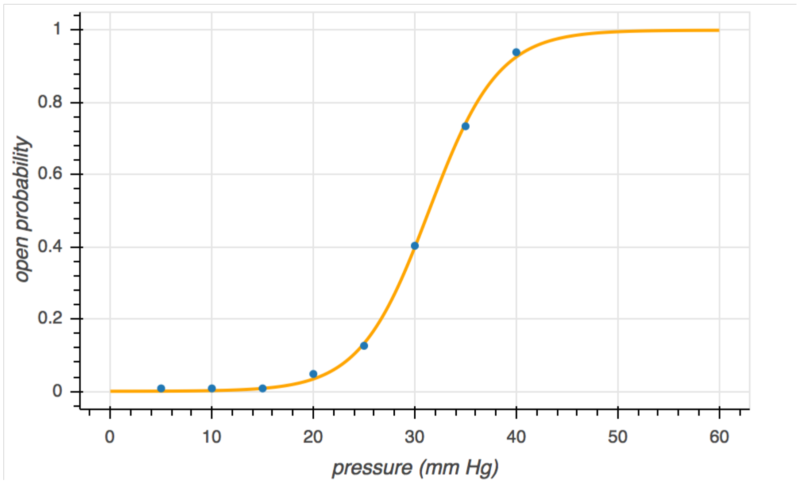

Figure 11: Curve fit of the Perozo, et al., patch clamp data.

Interestingly, we were able to obtain that absence tension on the membrane, the energy difference between the open and closed state is about 9 $k_B T$. This gives an open probability in the absence of tension of about 10^{-4}, which would be difficult to observe experimentally by just measuring current through an un-tensed channel. This also means that in the absence of tension, the channel is almost always closed. It takes tension to open it, hence the name mechanosensitive.

This exercise has shown the power of two-state models in helping to set up experiments to probe the physical nature of ion channels.

```python
import numpy as np
import scipy.optimize
import bokeh.plotting

# The data sets
pressure = np.array([5, 10, 15, 20, 25, 30, 35, 40])
p_open = np.array([0.008, 0.008, 0.008, 0.048,
                   0.126 ,0.403 ,0.734 ,0.939])

# Define theoretical p_open
def p_open_theor(pressure, beta_epsilon, beta_a):
    """Theoretical p_open"""
    return 1 / (1 + np.exp(beta_epsilon - beta_a * pressure))

# Define residuals
def resid(params, pressure, p_open):
    # Unpack parameters
    beta_epsilon, beta_a = params

    # Compute residuals
    return p_open - p_open_theor(pressure, beta_epsilon, beta_a)

# Bound on parameters, first lower bounds, then upper
bounds = ((-np.inf, 0), (np.inf, np.inf))

# Initial guess
p0 = np.array([0.1, 0.1])

# Perform least squares
res = scipy.optimize.least_squares(resid,
                                   p0,
                                   args=(pressure, p_open),
                                   bounds=bounds)

# Put out the optimal parameters
beta_epsilon, beta_a = res.x

# Generate smooth curve
pressure_smooth = np.linspace(0, 70, 200)
p_open_fit = p_open_theor(pressure_smooth, beta_epsilon, beta_a)

```

```
# Make the plot
p = bokeh.plotting.figure(plot_width=500,
                          plot_height=300,
                          x_axis_label='pressure (mm Hg)',
                          y_axis_label='open probability')
p.line(pressure_smooth, p_open_fit, line_width=2, color='orange')
p.circle(pressure, p_open)
bokeh.io.show(p)

# Report results
print("""Most probable fit parameters:βε
: {0:.2f}β
a: {1:.2f} (mm Hg)⊠¹""".format(beta_epsilon, beta_a))
```

perozo_regression.py