BE/APh 161: Physical Biology of the Cell

Justin Bois

Caltech

Winter, 2019

Donna and Benjamin M.
Rosen Bioengineering Center

This document was prepared at Caltech with financial support from the Donna and Benjamin M. Rosen Bioengineering Center.

6 Allostery and the Monod-Wyman-Changeux model

In a previous lecture, we used the theory of equilibrium statistical mechanics to study ligand receptor binding. We then applied a similar theoretical approach to treat a mechano-sensitive ion channel behavior. In this lecture, we extend that ligand-receptor binding theory to include more states beyond "bound" and "unbound." As we work through the theory, we will discover some of the basic ideas behind allostery and introduce the famous Monod-Wyman-Changeux (MWC) model.

6.1 Allostery

Consider an enzyme that has two binding sites. One site is involved in its activity, say with binding its target substrate. We will call this the active site. The other binding site binds some other ligand. Important, when this other site is bound, the activity of the active site is either positively or negatively affected. This phenomenon, where binding of one site of a protein or protein complex affects the activity of another is called **allostery**.

We can explore allostery using the same states-and-weights approach as with the vanilla ligand-receptor binding we have already studied. In that case, we had two states, bound and unbound. Now, we also specify whether or not the receptor is active of inactive. So, there are now four states, unbound and inactive, unbound and active, bound and inactive, and bound and active. Each of these four states has an energy associated with it.

It is more convenient to treat our system to be only the receptor and possibly the single ligand bound to it. In this case, the energy of the bound state is supplemented with the chemical potential associated with taking the ligand out of solution, as we showed in lecture 4. That is, we subtract $\mu = \mu_0 + k_B T \ln x$, where x is the mole fraction of ligand, from the energy to get the statistical weight. This is shown in the states and weights table below.

state	description	energy	statistical weight
	unbound, inactive	E_{ui}	$\mathrm{e}^{-\beta E_{\text{ui}}}$
	unbound, active	E_{ua}	$\mathrm{e}^{-\beta E_{\text{ua}}}$
	bound, inactive	E_{bi}	$x\mathrm{e}^{-\beta(E_{\text{bi}}-\mu_0)}$
	bound, active	E_{ba}	$x\mathrm{e}^{-\beta(E_{\text{ba}}-\mu_0)}$

We are most interested in the probability that the receptor is active, which we can compute from the states and weights table.

$$p_{\text{active}} = \frac{\text{sum of weights of active states}}{\text{sum of all weights}}$$

$$= \frac{\mathrm{e}^{-\beta E_{\text{ua}}} + x\mathrm{e}^{-\beta(E_{\text{ba}}-\mu_0)}}{\mathrm{e}^{-\beta E_{\text{ui}}} + \mathrm{e}^{-\beta E_{\text{ua}}} + x\mathrm{e}^{-\beta(E_{\text{bi}}-\mu_0)} + x\mathrm{e}^{-\beta(E_{\text{ba}}-\mu_0)}}. \tag{6.1}$$

This can be simplified be defining dissociation constants for ligand-receptor binding when the receptor is respectively in the inactive and active states,

$$K_{\text{di}} = \rho_{\text{H}_2\text{O}}\, \text{e}^{-\beta(E_{\text{ui}}+\mu_0-E_{\text{bi}})}, \tag{6.2}$$

$$K_{\text{da}} = \rho_{\text{H}_2\text{O}}\, \text{e}^{-\beta(E_{\text{ua}}+\mu_0-E_{\text{ba}})}, \tag{6.3}$$

where $\rho_{\text{H}_2\text{O}}$ is the number density of solvent. We can also use it to define the concentration of ligand as $c = \rho_{\text{H}_2\text{O}} x$. Then, the expression for the probability that the receptor is active is

$$\begin{aligned} p_{\text{active}} &= \frac{1 + c/K_{\text{da}}}{1 + c/K_{\text{da}} + \text{e}^{-\beta\,\Delta E_{\text{u}}}\left(1 + \frac{K_{\text{da}}}{K_{\text{di}}}(c/K_{\text{da}})\right)} \\ &= \frac{1 + c/K_{\text{da}}}{1 + c/K_{\text{da}} + \text{e}^{-\beta\Delta E_{\text{u}}} + \text{e}^{-\beta\Delta E_{\text{b}}}(c/K_{\text{da}})}, \end{aligned} \tag{6.4}$$

where $\Delta E_{\text{u}} = E_{\text{ui}} - E_{\text{ua}}$ is the difference in energies of the inactive and active states in the absence of ligand and $\Delta E_{\text{b}} = E_{\text{bi}} - E_{\text{ba}}$ is the difference in energies of the inactive and active states when the receptor is bound to ligand.

To understand this expression, we can consider the small and large c limits. In the small ligand concentration limit, we have

$$\text{small } c: \quad p_{\text{active}} = \frac{1}{1 + \text{e}^{-\beta\Delta E_{\text{u}}}}, \tag{6.5}$$

which is what we expect from a two-state model for receptor activity that does not include binding. We will consider this to be the base case of activity, that is the probability that the receptor is active in absence of ligand. In the limit of large ligand concentration, we have

$$\text{large } c: \quad p_{\text{active}} = \frac{1}{1 + \frac{K_{\text{da}}}{K_{\text{di}}}\,\text{e}^{-\beta\Delta E_{\text{u}}}} = \frac{1}{1 + \text{e}^{-\beta\Delta E_{\text{b}}}}. \tag{6.6}$$

So, if the ratio of the dissociation constants, $K_{\text{da}}/K_{\text{di}}$, is less than one, i.e., if the ligand binds more tightly to the active state than to the inactive state, the activity of the receptor is enhanced by the ligand. This is allostery; binding of a ligand at one site of an enzyme enhances activity at another.

To better visualize the how p_{active} varies with ligand concentration, see Fig. 13 for a plot.

It is also useful to quantify how effective allosteric activation can be compared to the base case of no ligands. The maximum fold change in activity compared to the base case if found by dividing the large c limit of p_{active} by the base case p_{active}.

$$\text{max fold change} = \frac{\text{large } c \text{ limit of } p_{\text{active}}}{\text{small } c \text{ limit of } p_{\text{active}}} = \frac{1 + \text{e}^{-\beta\Delta E_{\text{u}}}}{1 + \frac{K_{\text{da}}}{K_{\text{di}}}\,\text{e}^{-\beta\Delta E_{\text{u}}}} = \frac{1 + \text{e}^{-\beta\Delta E_{\text{u}}}}{1 + \text{e}^{-\beta\Delta E_{\text{b}}}}. \tag{6.7}$$

So, the maximum achievable fold change is set by $1 + \text{e}^{-\beta\Delta E_{\text{u}}}$. The larger the energy difference between the active and inactive unbound states, the more effective the ligand-mediated allosteric activation.

The Monod-Wyman-Changeux model. The example we just worked out is an example of a Monod-Wyman-Changeux (MWC) model. The main idea behind the MWC model is the presence of two states, whether or not ligand is bound, and that ligand can bind in either configuration. As we have seen, ligand binding shifts the equilibrium between the two states. It is a simple and beautiful idea, and we will come to see that it is very powerful and ubiquitous throughout cell biology.

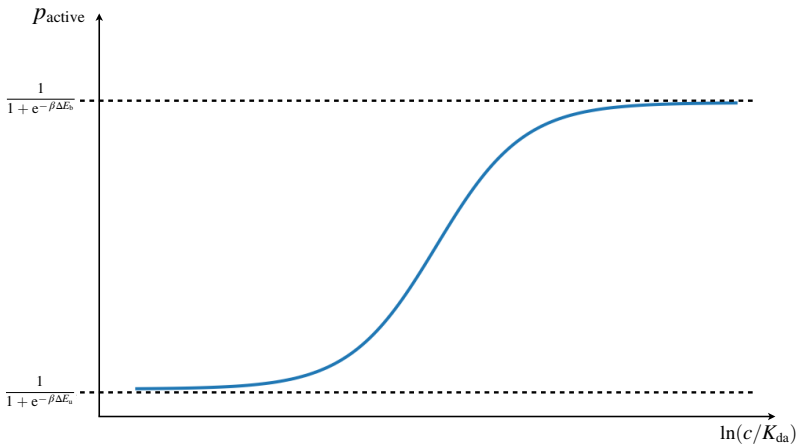

Figure 13: A sketch of the probability that the receptor is active as a function of ligand concentration.

6.2 Ligand-gated ion channels

In the last lecture, we considered the statistical mechanics of a mechano-sensitive ion channel. We will not turn to ion channels that are **ligand-gated**, and treat them using the MWC framework. That is, the ion channel has two states, open and closed, and the energetics of ligand binding in those two states varies.

In our model, we will assume that there are two binding sites for ligands on the channel. We may therefore have four binding states, no sites bound, site one bound, site two bound, and both sites bound. With the two states of the ion channel, open and closed, that leaves eight total states to enumerate. We will assume that both binding sites have the same energy, such that the single bound open states have the same energy, as do the singly bound closed states. We again use the convenient method of including the chemical potential of the ligand in the statistical weights so we do not need to explicitly count spatial configurational states of the ligand. With these considerations in mind, we can write the states-and-weights table.

state	energy	statistical weight
	$2E_{\mathrm{uc}}$	$\mathrm{e}^{-2\beta E_{\mathrm{uc}}}$
	$E_{\mathrm{uc}} + E_{\mathrm{bc}}$	$x\,\mathrm{e}^{-\beta(E_{\mathrm{uc}}+E_{\mathrm{bc}}-\mu_0)}$
	$E_{\mathrm{uc}} + E_{\mathrm{bc}}$	$x\,\mathrm{e}^{-\beta(E_{\mathrm{uc}}+E_{\mathrm{bc}}-\mu_0)}$
	$2E_{\mathrm{bc}}$	$x^2\,\mathrm{e}^{-2\beta(E_{\mathrm{bc}}-\mu_0)}$
	$2E_{\mathrm{uc}}$	$\mathrm{e}^{-2\beta E_{\mathrm{uo}}}$
	$E_{\mathrm{uo}} + E_{\mathrm{bo}}$	$x\,\mathrm{e}^{-\beta(E_{\mathrm{uo}}+E_{\mathrm{bo}}-\mu_0)}$
	$E_{\mathrm{uo}} + E_{\mathrm{bo}}$	$x\,\mathrm{e}^{-\beta(E_{\mathrm{uo}}+E_{\mathrm{bo}}-\mu_0)}$
	$2E_{\mathrm{bo}}$	$x^2\,\mathrm{e}^{-2\beta(E_{\mathrm{bo}}-\mu_0)}$

For the case of this ion channel, the "active state" is the open state. So, we wish to compute p_{open}. We directly read off the states and weights table to compute it.

$$
\begin{aligned}
p_{\mathrm{open}} &= \frac{\text{sum of weights of open states}}{\text{sum of all weights}} \\
&= \frac{\mathrm{e}^{-2\beta E_{\mathrm{uo}}} + 2x\,\mathrm{e}^{-\beta(E_{\mathrm{uo}}+E_{\mathrm{bo}}-\mu_0)} + x^2\,\mathrm{e}^{-2\beta(E_{\mathrm{bo}}-\mu_0)}}{\mathrm{e}^{-2\beta E_{\mathrm{uc}}} + 2x\,\mathrm{e}^{-\beta(E_{\mathrm{uc}}+E_{\mathrm{bc}}-\mu_0)} + x^2\,\mathrm{e}^{-2\beta(E_{\mathrm{bc}}-\mu_0)} + \mathrm{e}^{-2\beta E_{\mathrm{uo}}} + 2x\,\mathrm{e}^{-\beta(E_{\mathrm{uo}}+E_{\mathrm{bo}}-\mu_0)} + x^2\,\mathrm{e}^{-2\beta(E_{\mathrm{bo}}-\mu_0)}} \\
&= \frac{1 + 2x\,\mathrm{e}^{-\beta(E_{\mathrm{bo}}-E_{\mathrm{uo}}-\mu_0)} + x^2\,\mathrm{e}^{-2\beta(E_{\mathrm{bo}}-E_{\mathrm{uo}}-\mu_0)}}{1 + 2x\,\mathrm{e}^{-\beta(E_{\mathrm{bo}}-E_{\mathrm{uo}}-\mu_0)} + x^2\,\mathrm{e}^{-2\beta(E_{\mathrm{bo}}-E_{\mathrm{uo}}-\mu_0)} + \mathrm{e}^{-\beta\Delta E_{\mathrm{u}}}\left(1 + 2x\,\mathrm{e}^{-\beta(E_{\mathrm{bc}}-E_{\mathrm{uc}}-\mu_0)} + x^2\,\mathrm{e}^{-2\beta(E_{\mathrm{bc}}-E_{\mathrm{uc}}-\mu_0)}\right)} \\
&= \frac{(1 + c/K_{\mathrm{do}})^2}{(1 + c/K_{\mathrm{do}})^2 + \mathrm{e}^{-2\beta\Delta E_{\mathrm{u}}}\left(1 + \frac{K_{\mathrm{do}}}{K_{\mathrm{dc}}}(c/K_{\mathrm{do}})\right)^2} \\
&= \frac{(1 + c/K_{\mathrm{do}})^2}{(1 + c/K_{\mathrm{do}})^2 + (\mathrm{e}^{-\beta\Delta E_{\mathrm{u}}} + \mathrm{e}^{-\beta\Delta E_{\mathrm{b}}}(c/K_{\mathrm{do}}))^2}
\end{aligned}
\tag{6.8}
$$

where

$$\Delta E_{\mathrm{u}} = E_{\mathrm{uc}} - E_{\mathrm{uo}}, \tag{6.9}$$

$$\Delta E_{\mathrm{b}} = E_{\mathrm{bc}} - E_{\mathrm{bo}}, \tag{6.10}$$

$$K_{\mathrm{do}} = \rho_{\mathrm{H_2O}}\,\mathrm{e}^{-\beta(E_{\mathrm{uo}}+\mu_0-E_{\mathrm{bo}})}, \tag{6.11}$$

$$K_{\mathrm{dc}} = \rho_{\mathrm{H_2c}}\,\mathrm{e}^{-\beta(E_{\mathrm{uc}}+\mu_0-E_{\mathrm{bc}})}. \tag{6.12}$$

The functional form is similar to what we got in the allosteric ligand-receptor binding case, but with squared terms. The high and low ligand concentration limits are similar, except again with squared terms.

$$\text{small } c: \quad p_{\text{active}} = \frac{1}{1+\mathrm{e}^{-2\beta\Delta E_{\text{u}}}}, \tag{6.13}$$

$$\text{large } c: \quad p_{\text{active}} = \frac{1}{1+\left(\frac{K_{\text{do}}}{K_{\text{dc}}}\,\mathrm{e}^{-\beta\Delta E_{\text{u}}}\right)^2} = \frac{1}{1+\mathrm{e}^{-2\beta\Delta E_{\text{b}}}}. \tag{6.14}$$

We can thus determine the **dynamic range**, r, of the channel.

$$r = p_{\text{open}}^{\text{max}} - p_{\text{open}}^{\text{min}} = \frac{1}{1+\left(\frac{K_{\text{do}}}{K_{\text{dc}}}\,\mathrm{e}^{-\beta\Delta E_{\text{u}}}\right)^2} - \frac{1}{1+\mathrm{e}^{-2\beta\Delta E_{\text{u}}}}. \tag{6.15}$$

If we have N ion channels in a cell, the dynamic range of the entire cell is $r_{\text{cell}} = Nr$. The dynamic range is large for large ΔE_{u} (the energy of the closed state is much higher than that of the open state in the absence of ligand) and for small $K_{\text{do}}/K_{\text{dc}}$ (the ligands bind with much greater affinity to the open state).

6.2.1 The logistic equation and the Bohr parameter

The functional forms of the expressions for p_{active} in the allosteric receptor example and for p_{open} in the ligand-gated ion channel example are similar. In fact, we can re-write the functional form in terms of the logistic equation we have seen for two-state models. After all, these models are two-state models (active/inactive or open/closed); the added wrinkle is that ligand concentrations affect the probabilities of the respective states. For the ion channels, we can write

$$p_{\text{open}} = \frac{1}{1+\mathrm{e}^{-\beta F(c)}}, \tag{6.16}$$

a logistic equation,[5] where $F(c)$ is the **Bohr parameter**.[6] The Bohr parameter for the ligand-gated ion channel we have been considering is

$$F(c) = \Delta E_{\text{u}} + k_B T \ln\left(\frac{(1+c/K_{\text{do}})^2}{\left(1+\frac{K_{\text{do}}}{K_{\text{dc}}}(c/K_{\text{do}})\right)^2}\right). \tag{6.17}$$

Note that the Bohr parameter resembles the form of a chemical potential. The ligand-less two state model energy is adjusted by a correction related to the concentration of ligand and the respective binding energies.

6.2.2 Data collapse

Considering that all two-state models, including those modeled using MWC considerations, have an active (or open) probability given by the logistic equation, all p_{active} curves should fall on the same line

[5] Also called a Fermi-Dirac equation.

[6] The Bohr parameter is named after Christian Bohr, the father of Niels Bohr. He described what is now called the Bohr effect, in which presence of CO_2 decreases hemoglobin's oxygen binding efficiency. The Bohr parameter arises in that case as well.

when plotted against the Bohr parameter. So, if we could determine ΔE_u, K_{do}, and K_{dc} by performing experiments and statistical inference, we can compute the Bohr parameter $F(c)$ for each value of c. If we then plot the measured p_{active} versus $F(c)$, all points should fall along the logistic curve given by (6.17).

To investigate this, we will use data acquired in Henry Lester's lab on the nicotinic acetylcholine receptor/ion channel. This ion channel is perhaps the best studied ion channel in nature, certainly of importance in the human nervous system. Its structure is shown in Fig. 14. The experimenters performed voltage clamp experiments to get open probabilities of the ion channels as a function of ligand (in this case acetylcholine, abbreviated ACh). They performed mutations of the different domains of the receptor and repeated the experiments, showing different responses to ligand. Their original figure is shown in Fig. **??**.

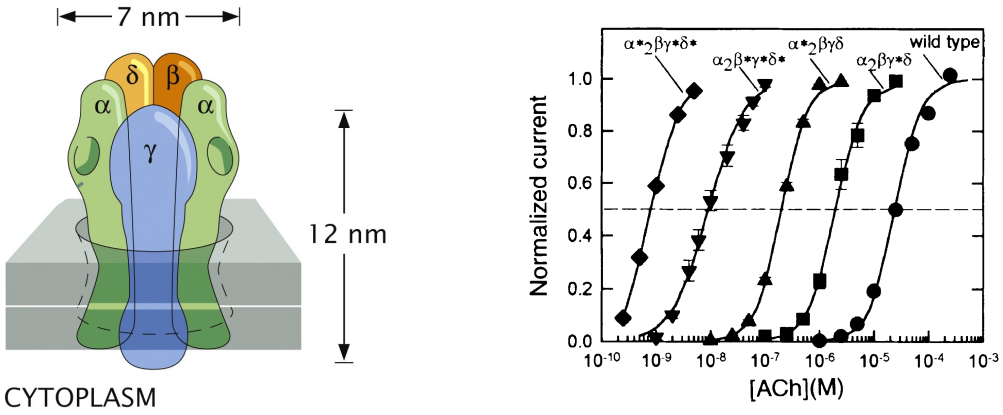

Figure 14: Left, a schematic of the nicotinic acetylcholine receptor and ion channel. Adapted from Fig. 7.26 of *PBoC2*. Right, voltage clamp experimental data for receptors containing various mutations. Figure taken from Labarca, et al., *Nature*, **376**, 514–516.

I digitized these data and performed a maximum likelihood estimate to get the necessary parameters. I then computed the Bohr parameter for each data point and plotted all data together on one plot. The result is shown in Fig. 15. The code to perform this analysis is at the end of this lecture.

6.3 Information and channel capacity

Ligand-gated ion channels sense the surroundings. If a channel is open, it is indicative that there are likely more ligands around than when it is closed. So, we may ask, how much *information* about the ligand concentration does the open or closed states of channels give the cell? Specifically, say we have N_{cell} ion channels in a cell and that n of them are open. What can we learn about the ligand concentration c given that we know n and N?

We have already dabbled in **information theory** when we derived the Boltzmann distribution. We will now apply these ideas to quantify how much information the channel state gives about the ligand concentration. That is, we seek the **mutual information** between the open-or-closed state of the channels and the ligand concentration.

The mutual information between two random variables X and Y is the entropy loss that is incurred by knowing Y.

$$I(X;Y) = S[X] - S[X \mid Y], \tag{6.18}$$

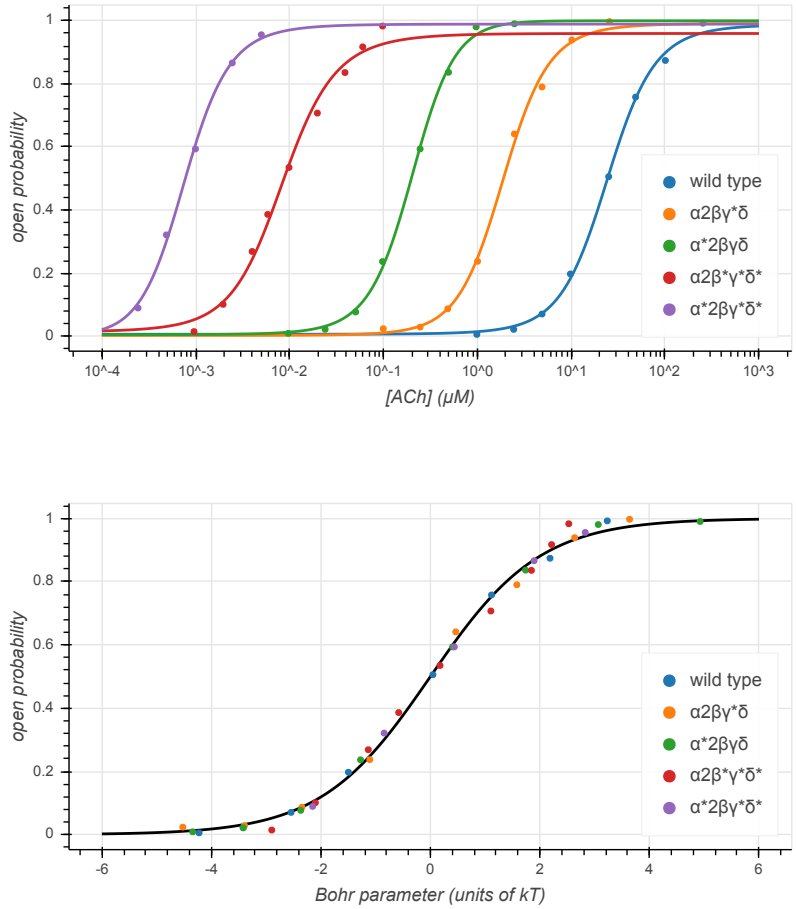

Figure 15: Top, results of maximum likelihood estimate curve fits of the data from Labarca, et al., *Nature*, **376**, 514–516. Bottom, all data sets plotted against the Bohr parameter. The logistic curve is shown in black.

where we have introduced the notion of **conditional entropy**,

$$S[X \mid Y] = \sum_y P(y) \left(-\sum_x P(x \mid y) \log_2 P(x \mid y) \right) . \tag{6.19}$$

The conditional entropy is then the entropy associated with the distribution $P(X \mid Y)$, averaged over Y. The mutual information is then

$$I(X;Y) = -\sum_x P(x) \log_2 P(x) + \sum_y P(y) \left(\sum_x P(x \mid y) \log_2 P(x \mid y) \right) . \tag{6.20}$$

It can be shown that the mutual information is symmetric, such that $I(X;Y) = I(Y;X)$.

In the present case, we take $X = c$ and $Y = n$. I will not work out the mathematical details here (see the Marzen and Phillips paper), but will state without proof that the maximum mutual information possible, called the **channel capacity**, is approximately (in the low noise limit)

$$I_{\text{opt}} \approx \log_2 \left(\frac{1}{\sqrt{2\pi e}} \int \mathrm{d}n \, \sigma_n \right) , \tag{6.21}$$

where σ_n is the standard deviation of $P(n \mid c, N)$. We know that $P(n \mid c, N)$ describes a Binomial distribution, where the probability that a given channel is open is given the expression we derived in (6.8). The standard deviation is then that for a Binomial distribution,

$$\sigma_n^2 = N p_{\text{open}}(1 - p_{\text{open}}). \tag{6.22}$$

Using this expression and evaluating the integral gives

$$I_{\text{opt}} \approx \log_2 \left(\sqrt{\frac{2N}{\pi e}} \left(\sin^{-1} \sqrt{p_{\text{open}}^{\text{max}}} - \sin^{-1} \sqrt{p_{\text{open}}^{\text{min}}} \right) \right), \tag{6.23}$$

which is related to the dynamic range, since the inverse sine function $\sin^{-1}(x)$ is monotonic on the interval $0 \leq x \leq 1$.

So, the bigger the dynamic range, the larger the channel capacity, and the more then cell and "know" about its surroundings. As we have seen, having multiple ligands bind to the channel to control the gating where binding it tighter when the channel is open, boosts the dynamic range, therefore increasing the channel capacity.

```
import numpy as np
import pandas as pd
import scipy.optimize
import bokeh.plotting
import bokeh.io

# Load in data set
df = pd.read_csv('lester_acetylcholine.csv')

# Get units in molar
df['[ACh] (M)'] *= 1e6

df = df.rename(columns={'[ACh] (M)': '[ACh] (µM)'})

# Set up data frame with MLE results
cols = ['Kd_open', 'Kd_closed', 'beta_deltaE', 'genotype']
df_best_fit = pd.DataFrame(columns=cols)


def p_open_theor(c, log_Kd_open, log_Kd_closed, beta_deltaE):
    """Theoretical curve for open probability"""
    Kd_open = np.exp(log_Kd_open)
    Kd_closed = np.exp(log_Kd_closed)
    a = (1 + c/Kd_open)**2
    b = (1 + c/Kd_closed)**2

    return a / (a + b * np.exp(-beta_deltaE))


def resid(params, c, p_open):
    """Residual from theoretical for use in least squares."""
    return p_open - p_open_theor(c, *params)


```

```
# Set up plots
p = bokeh.plotting.figure(plot_height=300,
                          plot_width=600,
                          x_axis_label='[ACh] (µM)',
                          y_axis_label='open probability',
                          x_axis_type='log')
p2 = bokeh.plotting.figure(plot_height=300,
                           plot_width=600,
                           x_axis_label='Bohr parameter (units of kT)',
                           y_axis_label='open probability')

# Theoretical logistic curve
F = np.linspace(-6, 6, 200)
p2.line(F, 1 / (1 + np.exp(-F)), color='black', line_width=2)


colors = bokeh.palettes.d3['Category10'][10]
Ach_smooth = np.logspace(-4, 3, 200)

# Initial guess for curve fits
p0 = np.array([-1, 0, -6])

for i, gtype in enumerate(df['genotype'].unique()):
    # Load in data for one genotype
    sub_df = df.loc[df['genotype']==gtype, :]
    c, p_open = sub_df['[ACh] (µM)'].values, sub_df['p_open'].values

    # Perform curve fit
    res = scipy.optimize.least_squares(resid, p0, args=(c, p_open))

    # Store results
    Kd_open, Kd_closed = np.exp(res.x[:2])
    beta_deltaE = res.x[2]
    df_res = pd.DataFrame(columns=cols,
                          data=[[Kd_open, Kd_closed, beta_deltaE,
                          gtype]])
    df_best_fit = df_best_fit.append(df_res, ignore_index=True)

    # Plot fits
    p.line(Ach_smooth,
           p_open_theor(Ach_smooth, *res.x),
           line_width=2,
           color=colors[i])
    p.circle(c, p_open, color=colors[i], legend=gtype)

    # Plot using Bohr parameter (data collapse)
    a = (1 + c/Kd_open)**2
    b = (1 + c/Kd_closed)**2
    F = beta_deltaE + np.log(a) - np.log(b)
    p2.circle(F, p_open, color=colors[i], legend=gtype)

```

```
p.legend.location = 'bottom_right'
p2.legend.location = 'bottom_right'

# Save as SVG
p.output_backend = 'svg'
p2.output_backend = 'svg'
bokeh.io.export_svgs(p, filename='lester_mle.svg')
bokeh.io.export_svgs(p2, filename='lester_data_collapse.svg')
```

lester_curves.py