Bi 1x
Efflux pumps

1 Overview

In this lab, we will investigate antibiotic resistance and will work on an active research project! The question we are trying to answer is how a specific sequence in the genome controls how resistant bacteria are to a certain antibiotic, and how mutations in this sequence can either increase or decrease the growth rate of the bacteria. To do that, we will build mathematical models for different processes in the cell, and put them together in order to connect measurements of growth rates using a plate reader with the sequences in the genome.

First, we will build a model that predicts the growth rate of a cell given the level of expression of a gene that expresses a protein that can pump out the antibiotic out of the cell. The more pumps there are, the more antibiotic can be pumped out, and the faster the cell can grow.

Second, we will build a model that connects the sequence an element in the genome to the level of expression of the gene. This element is a promoter sequence to which RNA polymerase binds to make the mRNA coding for the gene. If the promoter and the RNA polymerase have weak binding, less mRNA is produced, and therefore fewer copies of the protein.

While both of these approaches have been studied separately before, the novelty of our experiment is to connect the two models, ultimately allowing us to predict the growth rate of the bacteria from one single sequence. If we succeed, we created a piece of knowledge that no one has learned before!

2 Background

In this section, we will cover the basics of regulation gene expression as well as give a more detailed explanation of our experimental system.

2.1 From sequence to gene expression

What makes a cone cell in the retina different from a skin cell? Both have the same set of genes, but clearly they differ substantially in both structure and function. The reason, in part, is that although both cells share the same genes, they have different profiles of *gene expression*. The question of how gene expression is regulated is a major area of inquiry in contemporary biology, both because of its importance to biological function and because of the richness and diversity of gene regulation strategies that can be found in nature. Furthermore, a quantitative understanding

of regulation of gene expression is necessary for engineering applications. Regulation occurs at all steps along the central dogma, from methylation of genomic DNA (known as *epigenetics*) to the chemical modifications made to proteins after translation that modulate their function or localization.

As such, there are many different players that can be involved in regulating the expression of a gene. Here, we are only considering the **RNA polymerase** (RNAP). RNAP is recruited to the transcription start site (TSS) of a gene by a **sigma factor**. A sigma factor recognizes a specific target sequence in the genome; this sequence is called a **promoter** sequence. The probability of initiation of transcription is higher if the promoter sequence and target sequence of the sigma factor are highly similar. One part of the model we are exploring in this experiment is how differences in the promoter sequence lead to various levels of expression of the downstream gene.

2.2 Antibiotic resistance

Antibiotic compounds are often small molecules that bind important proteins in the cell and inhibit essential functions, leading to stalling of growth (bacteriostatic antibiotics) or even kill the bacteria (bactericidal antibiotics). Most of these molecules are actually produced by microbes as a way of fighting off competition in the environment. As a defense mechanism, cells can evolve different ways of inhibiting the effect of an antibiotic. Random mutation of genes coding for protein targets of an antibiotic can suppress antibiotic-target binding, thereby rendering the antibiotic impotent. Other mechanisms revolve around reducing the concentration of the antibiotic inside of the cell, e.g., by pumping the molecule out of the cell. In this module, we will explore this mode of resistance against the drug tetracycline. Tetracycline binds the ribosome, stalling protein synthesis. There is resistance gene, *tetA*, which codes for a **secondary efflux pump**. This means that it exchanges a tetracycline molecule against a chemical gradient, which in this case is a hydrogen gradient. In this module, will derive a mathematical model for the concentration of the drug and how it changes as a function of expression levels of *tetA*.

2.3 Model for bacterial growth in the presence of antibiotic

In this section, we derive expressions for the growth rate of bacteria in the presence of antibiotics based on known physiological interactions between cell membrane and ribosomes with tetracycline. A sketch of these interactions is shown in Fig. 1. Tetracycline can be exchanged in and our of the cell membrane. Once inside a cell, it binds ribosomes rendering them ineffective for protein production. To combat this, the efflux pump TetA actively pumps tetracycline out of the cell.

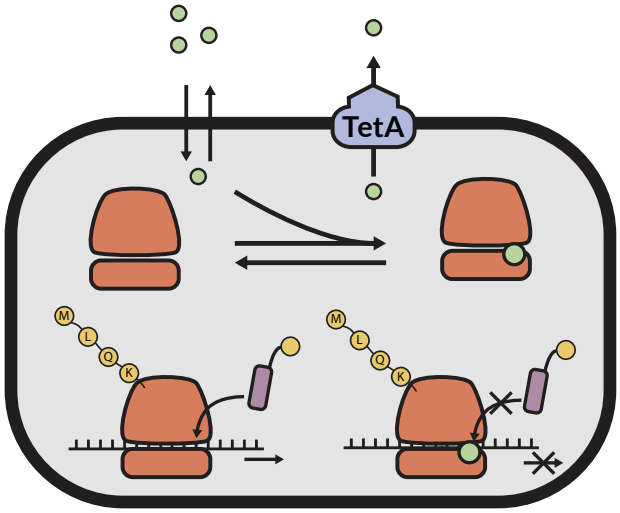

Figure 1: Mode of action for tetracycline and the efflux pump TetA. Tetracycline enters the cell passively and binds the small subunit of the ribosome, where it inhibits translation by blocking binding from the charged tRNA. TetA reduces the intracellular concentration of tetracycline by pumping out the drug against a proton gradient.

2.3.1 Empirical bacterial growth laws

In recent years, there has been a tremendous advancement in our understanding of bacterial physiology from a theoretical perspective. The group of Terrence Hwa has proposed and tested a series of empirical growth laws based on experimental observations of cellular behavior [5, 4]. A major observation is a law that describes a linear relationship between the concentration of ribosomes that are unbound to antibiotic, r_u, and the growth rate λ of the cell[1],

$$\lambda = \begin{cases} (r_u - r_{\min})\,\kappa_t & \text{for } r_u > r_{\min} \\ 0 & \text{for } r_u \leq r_{\min}. \end{cases} \tag{1}$$

where $r_{\min}$ is the minimal unbound ribosome concentration needed for growth and κ_t is an empirically determined proportionality factor called **translational capacity**. (Henceforth, for concision of language, we refer to ribosomes that are not bound to antibiotic as "unbound ribosomes." Naturally, these unbound ribosomes may be bound to mRNA.) This equation says that a minimal number of ribosomes is needed for growth (since some fraction of the ribosomal activity is allocated for maintaining protein levels in the cell), and that faster growth rate necessitates more ribosomes.

[1]We recognize that in the *E. coli* growth module, we referred to the growth rate as r, and in Neidhardt's paper, the growth rate was k. Now we are using λ. This may seem like a notational jumble, but we are keeping notation consistent with the papers that developed the models we are using in this module.

For convenience going forward, we will simply write this as

$$\lambda = (r_u - r_{\min})\,\kappa_t, \tag{2}$$

with the understanding that $r_u < r_{\min}$ results in zero (not negative) growth.

Though we will not use it in what follows, another result of their study is worth mentioning. The observed cells responded to translation inhibiting drugs by increasing the total (bound and unbound) ribosome concentration, r_{tot}. This relationship was also found to be linear,

$$\lambda = (r_{\max} - r_{\text{tot}})\,\kappa_n, \tag{3}$$

where $r_{\max}$ is the maximum possible total ribosome concentration in the cell and κ_n is the phenomenological constant of proportionality, referred as the nutritional capacity. This equation might seem at face confusing. It says that the growth rate *decreases* as the ribosomal fraction increases, apparently contrary to equation (2). Importantly, equation (2) relates the growth rate to *unbound* ribosomes and equation (3) related the growth rate to *total* ribosomes. In the presence of antibiotic, we can have $r_u \ll r_{\text{tot}}$, meaning that most ribosomes are bound to antibiotic and therefore cannot make protein. As antibiotic concentration gets really high, the cell cannot make more than $r_{\max}$ ribosomes (that is, r_{tot} can never exceed $r_{\max}$), and growth stops.

2.3.2 Dynamics of intracellular antibiotic concentration

We will now consider the respective processes that affect the intracellular antibiotic concentration a.

Inflow and outflow of antibiotic. Tetracycline enters the cell by passive diffusion through the outer membrane and subsequently via energy-dependent transport through the cytoplasmic membrane [3]. The net inflow of tetracycline into the cell by these processes is

$$q(a_{\text{ex}}, a) = P_{\text{in}}\, a_{\text{ex}} - P_{\text{out}}\, a, \tag{4}$$

where a_{ex} is the extracellular concentration of antibiotic and P_{in} and P_{out} are rate constants describing respectively the effective permeabilities for influx and outflux of antibiotic. Cells gain resistance by expressing antibiotic-specific secondary **efflux pumps** (sometimes called antiporters), which draw energy from a chemical gradient. In the case of the tetracycline antiporter, the antibiotic is exported against a proton gradient. We assume that the proton gradient is constant for the duration of the experiment. We can model the outflow of antibiotic due to efflux pumps with a **Michaelis-Menten** expression,

$$q_{\text{ex}}(a) = q_{\text{ex},0}\,\frac{a/K_{\text{M}}}{1 + a/K_{\text{M}}}, \tag{5}$$

where K_{M} is the Michaelis constant of the efflux pump and $q_{\mathrm{ex},0}$ is the maximum efflux rate (in units of concentration per time) which depends on the expression of the resistance gene.[2] In this model we include growth mediated feedback to gene expression [4]. That is, if growth rate is reduced, so is the expression of genes in the cell [1].

$$q_{\mathrm{ex},0} = \frac{\lambda}{\lambda_0} q_{\mathrm{ex},0}^{\mathrm{max}}, \tag{6}$$

where $q_{\mathrm{ex},0}^{\mathrm{max}}$ is the flow rate when antibiotic is removed so effectively that it does not impede growth. (A detailed discussion of $q_{\mathrm{ex},0}^{\mathrm{max}}$ and how it depends on regulation of the resistance gene is given in section 2.3.6.) Thus, we have

$$q_{\mathrm{ex}}(a) = q_{\mathrm{ex},0}^{\mathrm{max}} \frac{\lambda}{\lambda_0} \frac{a/K_{\mathrm{M}}}{1 + a/K_{\mathrm{M}}}. \tag{7}$$

Binding equilibrium between the ribosome and antibiotic. Tetracycline binds reversibly to the ribosome. We can write this as

$$\mathrm{R}_b \rightleftharpoons \mathrm{R}_u + \mathrm{A}, \tag{8}$$

where $\mathrm{R_b}$ is a bound ribosome. We will denote the total concentration of antibiotic-bound ribosomes as r_b, where $r_u + r_b = r_{\mathrm{tot}}$. We assume that establishing equilibrium for this binding happens much faster than other pertinent processes such that we can take the ribosome binding-unbinding reaction to be in equilibrium at all times such that

$$K_{\mathrm{d}} = \frac{r_u\, a}{r_b}. \tag{9}$$

Dilution due to growth. Finally, we note that as cells grow, by virtue of the total cellular volume increasing, the concentration of any species inside a cell decreases linearly with growth rate. E.g., antibiotic in the cell gets diluted with rate λa.

Dynamics of antibiotic. We can now write down the rate of change of the intracellular antibiotic concentration by summing up all of the processes we have consider.

$$\frac{\mathrm{d}a}{\mathrm{d}t} = \text{dilution via growth}$$

[2] If you are unfamiliar with Michaelis-Menten kinetics, do not worry. Importantly, at low antibiotic concentration relative to the Michaelis constant, q_{ex} increases linearly with a, but at high antibiotic concentration, q_{ex} reaches a maximum value. That is, the efflux pumps have an operational speed limit.

$$+ \text{ net gain due to in/outflow through the membrane}$$

$$+ \text{ loss due to efflux pumping out of the cell.} \tag{10}$$

Plugging in the respective mathematical expressions for each of these,

$$\frac{\mathrm{d}a}{\mathrm{d}t} = -\lambda a + P_{\text{in}}\, a_{\text{ex}} - P_{\text{out}}\, a - q_{\text{ex},0}^{\max} \frac{\lambda}{\lambda_0} \frac{a/K_{\text{M}}}{1 + a/K_{\text{M}}}. \tag{11}$$

2.3.3 Steady state growth rate

At long times, the system tends toward a **steady state**, a set of variables where all time derivatives vanish. We specifically want to find the steady-state growth rate λ as a function of the extracellular antibiotic concentration a_{ex} and the relevant physical parameters. At steady state, we have

$$\frac{\mathrm{d}a}{\mathrm{d}t} = -\lambda a + P_{\text{in}}\, a_{\text{ex}} - P_{\text{out}}\, a - q_{\text{ex},0}^{\max} \frac{\lambda}{\lambda_0} \frac{a/K_{\text{M}}}{1 + a/K_{\text{M}}} = 0. \tag{12}$$

This is a useful equation, in that it relates the growth rate λ to the extracellular antibiotic concentration a_{ex} and the physical parameters P_{in}, P_{out}, λ_0, K_{M}, and $q_{\text{ex},0}^{\max}$. However, it still contains the unknown variable a. So, we need to be able to write a in terms of λ and physical parameters.

To do so, we recall that the empirical law relating growth rate to ribosome, equation (2), which we may write as

$$r_u = r_{\min} + \frac{\lambda}{\kappa_t}. \tag{13}$$

Recall also the equilibrium expression for ribosome-antibiotic binding, equation (9), which we may write as

$$a = K_{\text{d}} \frac{r_b}{r_u}. \tag{14}$$

We know that the total ribosome concentration has to be the sum of bound and unbound ribosomes, $r_{\text{tot}} = r_b + r_u$, such that $r_b = r_{\text{tot}} - r_u$. Substituting this expression into our expression for a gives

$$a = K_{\text{d}} \frac{r_{\text{tot}} - r_u}{r_u}. \tag{15}$$

Let us return now to the empirical relation. We know that in the absence of antibiotic, $\lambda = \lambda_0$ and $r_u = r_{\text{tot}}$. Therefore,

$$r_{\text{tot}} = r_{\min} + \frac{\lambda_0}{\kappa_t}, \tag{16}$$

such that

$$r_{\text{tot}} - r_u = \frac{\lambda_0 - \lambda}{\kappa_t}. \tag{17}$$

Substituting into equation (15), we have

$$a(\lambda) = K_{\text{d}} \frac{(\lambda_0 - \lambda)/\kappa_t}{r_{\min} + \lambda/\kappa_t} = K_{\text{d}} \frac{\lambda_0 - \lambda}{\kappa_t r_{\min} + \lambda} \tag{18}$$

We now have an expression for a and a function of λ. We can insert this expression into equation (12) to get an equation that includes only λ and physical parameters. We will do that in the next section when we write everything in dimensionless form.

2.3.4 Nondimensionalization

To gain intuition and to simplify expressions, it is useful to put our expressions in dimensionless form. That is, we write the differential equation in terms of dimensionless variables and parameters

$$\tilde{t} = \lambda t, \tag{19}$$

$$\tilde{a} = a/K_{\text{d}}, \tag{20}$$

$$\tilde{a}_{\text{ex}} = a_{\text{ex}}/K_{\text{d}}, \tag{21}$$

$$\tilde{\lambda} = \lambda/\lambda_0, \tag{22}$$

$$\tilde{P}_{\text{in}} = P_{\text{in}}/\lambda_0, \tag{23}$$

$$\tilde{P}_{\text{out}} = P_{\text{out}}/\lambda_0, \tag{24}$$

$$\tilde{q}^{\max}_{\text{ex},0} = q^{\max}_{\text{ex},0}/K_{\text{d}}\,\lambda_0, \tag{25}$$

$$K = K_{\text{M}}/K_{\text{d}}, \tag{26}$$

$$\kappa = \kappa_t r_{\min}/\lambda_0. \tag{27}$$

This effectively means that we are choosing the units of time to be in terms of the inverse growth rate and the units of a (and a_{ex}) to be the dissociation constant K_{d}. In this case, we have

$$\frac{\text{d}\tilde{a}}{\text{d}\tilde{t}} = -\tilde{\lambda}\,\tilde{a} + \tilde{P}_{\text{in}}\,\tilde{a}_{\text{ex}} - \tilde{P}_{\text{out}}\,\tilde{a} - \tilde{q}^{\max}_{\text{ex},0}\,\tilde{\lambda}\,\frac{\tilde{a}/K}{1+\tilde{a}/K} = 0, \tag{28}$$

$$\tilde{a} = \frac{1-\tilde{\lambda}}{\kappa+\tilde{\lambda}}. \tag{29}$$

This identifies five dimensionless parameters that determine the growth rate. Three of these are rate parameters associated with transport of antibiotic in and out of the cell. First. $\tilde{P}_{\text{in}}$ is the ratio of the constant for intake, $\tilde{P}_{\text{out}}$, and $\tilde{q}_{\text{ex},0}^{\max}$. The parameter κ is the ratio of scale of the rate of translation $\kappa_t r_{\min}$ to the maximal growth rate λ_0. The parameter K is the ratio of the Michaelis constant for antibiotic transport out of the cell and the dissociation constant for binding antibiotic to the ribosome. The smaller κ is, the greater the resistance to the antibiotic.

We now substitute the expression for $\tilde{a}(\tilde{\lambda})$ and simplify to get

$$\frac{\mathrm{d}\tilde{a}}{\mathrm{d}\tilde{t}} = -\tilde{\lambda}\,\frac{1-\tilde{\lambda}}{\kappa+\tilde{\lambda}} + \tilde{P}_{\text{in}}\,\tilde{a}_{\text{ex}} - \tilde{P}_{\text{out}}\,\frac{1-\tilde{\lambda}}{\kappa+\tilde{\lambda}} - \tilde{q}_{\text{ex},0}^{\max}\,\tilde{\lambda}\,\frac{1-\tilde{\lambda}}{1+\kappa K-(1-K)\tilde{\lambda}}. \tag{30}$$

Finally, we use the chain rule to note that

$$\frac{\mathrm{d}\tilde{a}}{\mathrm{d}\tilde{t}} = \frac{\mathrm{d}\tilde{a}}{\mathrm{d}\tilde{\lambda}}\,\frac{\mathrm{d}\tilde{\lambda}}{\mathrm{d}\tilde{t}} = -\frac{1+\kappa}{(\kappa+\tilde{\lambda})^2}\,\frac{\mathrm{d}\tilde{\lambda}}{\mathrm{d}\tilde{t}}, \tag{31}$$

which gives our final differential equation for the (dimensionless) growth rate as a function of the external antibiotic concentration and physical parameters.

$$\frac{\mathrm{d}\tilde{\lambda}}{\mathrm{d}\tilde{t}} = \frac{(\kappa+\tilde{\lambda})^2}{1+\kappa}\left(\begin{array}{l}\tilde{\lambda}\,\dfrac{1-\tilde{\lambda}}{\kappa+\tilde{\lambda}} - \tilde{P}_{\text{in}}\,\tilde{a}_{\text{ex}} + \tilde{P}_{\text{out}}\,\dfrac{1-\tilde{\lambda}}{\kappa+\tilde{\lambda}} \\ \\ +\,\tilde{q}_{\text{ex},0}^{\max}\,\tilde{\lambda}\,\dfrac{1-\tilde{\lambda}}{1+\kappa K-(1-K)\tilde{\lambda}}\end{array}\right). \tag{32}$$

We seek the value(s) of $\tilde{\lambda}$ such that $\mathrm{d}\tilde{\lambda}/\mathrm{d}t = 0$. Note that $0 \le \tilde{\lambda} \le 1$.

2.3.5 Steady state growth rate(s)

To solve for the steady state growth rate, we need to find a value of λ such that $\mathrm{d}\tilde{\lambda}/\mathrm{d}t = 0$. This can be done by noting that setting the parenthetical term in equation (32) to zero results in $\mathrm{d}\tilde{\lambda}/\mathrm{d}t = 0$. Multipying the parenthetical term by $(\kappa+\tilde{\lambda})(1+\kappa K-(1-K)\tilde{\lambda})$ and setting the result to zero results in a cubic polynomial root finding problem,

$$\begin{aligned}0 = {} & (K+\tilde{q}_{\text{ex},0}^{\max}-1)\,\tilde{\lambda}^3 \\ & +(2-(1-K)(\tilde{P}_{\text{in}}+\tilde{P}_{\text{out}})-(1-\kappa)(\tilde{q}_{\text{ex},0}^{\max}+K))\,\tilde{\lambda}^2 \\ & -(1+\kappa(\tilde{q}_{\text{ex},0}^{\max}+K)-(2-(1-\kappa)K)\tilde{P}_{\text{out}}-(1-(1-2K)\kappa)\tilde{P}_{\text{in}}\,\tilde{a}_{\text{ex}})\,\tilde{\lambda} \\ & +(1+\kappa K)(\kappa\,\tilde{P}_{\text{in}}\tilde{a}_{\text{ex}}-\tilde{P}_{\text{out}}).\end{aligned} \tag{33}$$

There is an analytical solution, but it is the solution to a cubic equation and is a mess. We will instead solve for λ numerically, noting that if there is no solution with $0 \leq \tilde{\lambda} \leq 1$, then $\lambda = 0$ and the cells do not grow. We will have an in-class tutorial on how to do this.

In the meantime, it is worth analyzing the expression for $\mathrm{d}\tilde{\lambda}/\mathrm{d}t$. In Fig. 2, we see a plot of the time derivative of the growth rate as a function of the growth rate for parameters given in Table 2 in the next section, assuming a growth rate of 0.7 hr^{-1}, $a_{\mathrm{ex}} = 1$ µM, and $q_{\mathrm{ex},0}^{\mathrm{max}} = 11.9\ \mathrm{min}^{-1}$.

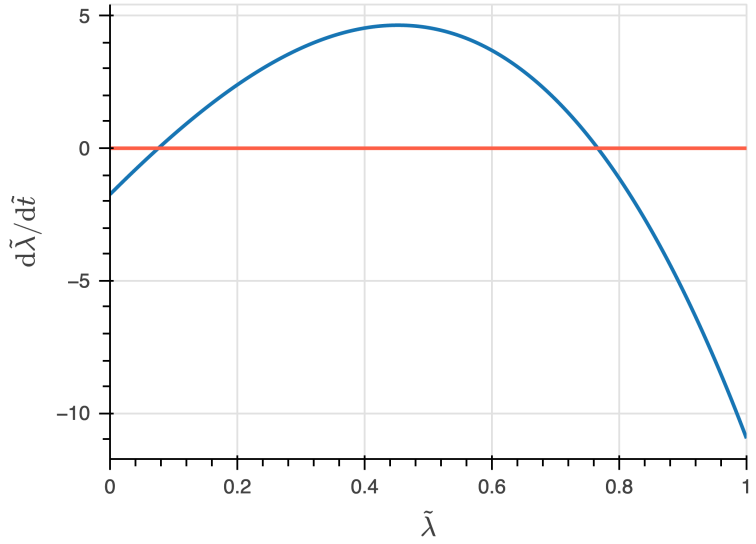

Figure 2: $\mathrm{d}\tilde{\lambda}/\mathrm{d}\tilde{t}$ versus $\tilde{\lambda}$ for parameters given in Table 2, assuming a growth rate of 0.7 hr^{-1}, $a_{\mathrm{ex}} = 1$ µM, and $q_{\mathrm{ex},0}^{\mathrm{max}} = 11.9\ \mathrm{min}^{-1}$.

Note that there are *two* steady states for these parameter values. Consider first the higher steady state, which is to the right. Let's say that cells in these conditions are growing at this growth rate (about $0.77\lambda_0$). Now say there is a perturbation where the growth rate increases slightly. To the right of that steady state, the derivative is negative, so the growth rate will slow back to the steady state. Similarly, if there is a perturbation where the cells decrease their growth rate, the time derivative of the growth rate is positive, so the cells grow faster and return to the steady state. This steady state is said to be **stable**.

Now consider the left steady state, which has a growth rate of about $0.075\lambda_0$. A perturbation to the right, where cells grow slightly faster, results in a positive time derivative of growth rate. As a result, the cells grow faster and faster until they reach the right steady state. Conversely, if there is a perturbation to the left, where cells grow slightly slower, the time derivative of the growth rate is negative, and the growth slows all the way to zero. Thus, the left steady state is **unstable**. Though the time derivative vanishes there, any perturbation pushes the cell population to either no growth or to the other steady state.

So, under these conditions, it is possible to have two populations of cells, those that do not grow (and essentially die), and those that grow at about 77% of the maximal growth rate.

There are not always two steady states. There can be zero, one, two, or three! Remember that when there are no steady states, there is no growth; the antibiotic concentration is too high. You can explore these possibilities for different parameter values at the interactive plot found here.

2.3.6 Model for the number of efflux pumps

The strains we use in this experiment all have a *tetA* gene, which codes for efflux pumps, in their genomes (except for the wild type strain MG1655, which has no *tetA* gene). Here, we will build a model for expression of this gene. There are no transcription factors that regulate the expression of *tetA*; its expression depends entirely the strength of the binding of RNA polymerase to its promoter sequence. Each of the strains we will work with has a different promoter sequence, and therefore a different strength of binding.

To model expression of this gene, and therefore to model the number of efflux pumps we may expect in a given cell, we will assume that the number of efflux pumps is proportional to the probability that polymerase can bind the promoter of the *tetA* gene. That is,

$$\text{number of efflux pumps} \propto p_{\text{bound}}, \tag{34}$$

where p_{bound} is the probability that the promoter region of the *tetA* gene has an RNA polymerase bound to it.

Say there are P RNAPs in the cell. Typically, all polymerases are bound to the genome. We consider a **state** to be the positions where all P of the polymerases are bound. Two states, one with and one without the promoter of interest being bound, are shown in Fig. 3.

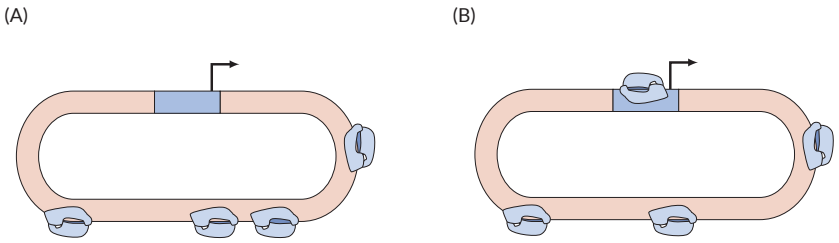

Figure 3: Two possible states of RNAP bound to the genome. (A) All P RNAP are bound outside the promoter. (B) One RNAP is bound to the promoter sequence, while all other $P-1$ RNAPs are bound unspecifically.

We assume that the binding energy for the site of interest is E, and that for any other site on the genome is E_0. The probability of a given state is given by the **Boltzmann weight**.

$$\text{probability of state } i \propto \mathrm{e}^{-E_i/k_\mathrm{B}T}, \tag{35}$$

where k_B is the **Boltzmann constant** and T is the temperature. Any state without a polymerase bound to the promoter has energy PE_0, since there are P polymerases bound to the genome, each with energy E_0. Similarly, any state with a polymerase bound to the promoter has energy $(P-1)E_0+E$. So, letting N_b be the number of states with a polymerase bound to the promoter and N_u be the number of states without a polymerase bound to the promoter, the probabilty of being bound is

$$p_\text{bound} = \frac{N_b\,\mathrm{e}^{-((P-1)E_0+E)/k_\mathrm{B}T}}{N_b\,\mathrm{e}^{-((P-1)E_0+E)/k_\mathrm{B}T} + N_u\,\mathrm{e}^{-PE_0/k_\mathrm{B}T}}. \tag{36}$$

If there are a total of N nonspecific binding sites on the genome (that is sites that are not the promoter), the the number of sites with the polymerase unbound is equal to the number of ways to distribute the polymerase on those N sites, which is

$$N_u = \binom{N}{P} = \frac{N!}{(N-P)!\,P!}. \tag{37}$$

Similarly,

$$N_B = \binom{N}{P-1} = \frac{N!}{(N-P+1)!\,(P-1)!}. \tag{38}$$

So, we have

$$p_\text{bound} = \frac{\frac{N!}{(N-P+1)!\,(P-1)!}\,\mathrm{e}^{-((P-1)E_0+E)/k_\mathrm{B}T}}{\frac{N!}{(N-P+1)!\,(P-1)!}\,\mathrm{e}^{-((P-1)E_0+E)/k_\mathrm{B}T} + \frac{N!}{(N-P)!\,P!}\,\mathrm{e}^{-PE_0/k_\mathrm{B}T}}. \tag{39}$$

Dividing both the numerator and denominator by the second term in the denominator gives

$$p_\text{bound} = \frac{\frac{P}{N-P+1}\,\mathrm{e}^{-(E-E_0)/k_\mathrm{B}T}}{1+\frac{P}{N-P+1}\,\mathrm{e}^{-(E-E_0)/k_\mathrm{B}T}}. \tag{40}$$

Finally, we note that since $N \gg P$, $N-P+1 \approx N$, giving

$$p_\text{bound} = \frac{\frac{P}{N}\,\mathrm{e}^{-(E-E_0)/k_\mathrm{B}T}}{1+\frac{P}{N}\,\mathrm{e}^{-(E-E_0)/k_\mathrm{B}T}}. \tag{41}$$

So, we have found a proportionality relationship for the number of efflux pumps.

$$\text{number of efflux pumps} \propto \frac{\frac{P}{N}\,\mathrm{e}^{-(E-E_0)/k_\mathrm{B}T}}{1+\frac{P}{N}\,\mathrm{e}^{-(E-E_0)/k_\mathrm{B}T}}. \tag{42}$$

Presumably, $q_{\text{ex},0}^{\max}$ is proportional to the number of efflux pumps, so that

$$q_{\text{ex},0}^{\max} \propto \frac{\frac{P}{N} \mathrm{e}^{-(E-E_0)/k_{\mathrm{B}}T}}{1 + \frac{P}{N} \mathrm{e}^{-(E-E_0)/k_{\mathrm{B}}T}}. \tag{43}$$

We are using 5 strains in this experiment, one of them being wild-type *E. coli*, which does not express TetA. The other four strains have different promoters sequences, for which the binding energies have been measured [2]. The strain names and the corresponding binding energies can be found in Table 1.

Table 1: Promoter binding energies

Strain	$E - E_0$ **in units of** $k_{\mathrm{B}}T$
MG1655	∞ (because there is no *tetA* gene)
3.19	-3.19
IW	-4.28
WTlac	-5.19
lacUV5	-7.93

2.3.7 Analysis of experimental data

In your experiment, you will vary the tetracycline concentration to impose various values of a_{ex} for various strains of bacteria. You will then measure the bacterial growth rate by growing bacteria and measuring absorbance over time using the Bi 1x plate reader as you did in the *E. coli* growth experiment. As a result, you will have a data set for each strain of bacteria consisting of (a_{ex}, λ) pairs. You will also do this in the absence of tetracycline in order to get the parameter λ_0. You will then use your (a_{ex}, λ) data to perform a regression to obtain the parameter $q_{\text{ex},0}^{\max}$. We have estimates for the rest of the parameters from the literature. A summary of the parameter values are shown below.

Table 2: Parameter values

Parameter	**Value**	**Reference**
$r_{\min}$	19.3 µM	Scott, et al., 2010
κ_t	0.061 $\mu\mathrm{M}^{-1} \cdot \mathrm{h}^{-1}$	Scott, et al., 2010
P_{in}	2.85 h^{-1}	Greulich, et al., 2015
P_{out}	2.85 h^{-1}	Greulich, et al., 2015
K_{d}	1 µM	Deris, et al., 2013
K_{M}	10 µM	Deris, et al., 2013
λ_0	measured	—
$q_{\text{ex},0}^{\max}$	determined by regression	—

You will then plot your measured $q_{\text{ex},0}^{\max}$ versus the binding energies (or a function thereof) to see if our proportionality relationship between $q_{\text{ex},0}^{\max}$ and the binding energies given by equation (43) holds.

3 Protocol

3.1 Growth in a Plate Reader

Here we are essentially running the same plate reader protocol that we have used for the *E. coli* growth protocol.

3.2 Well Plate Layout

We are going to test concentrations of antibiotic over multiple orders of magnitude. The concentration of the tetracycline hydrochloride stock we are using is 10 mg/mL in ethanol. (Note that tetracycline hydrochloride has a molar mass of 480 g/-mol.) There will be dilutions available at factors of $10\times$, $100\times$, and $1000\times$ dilutions of this solution. Because the stock contains ethanol, the dilutions were prepared to have as little ethanol as possible.

Each pair of lab partners will have their own specific concentration of antibiotic to use in the experiment. You will be assigned a team number in lab and will make 3.5 mL solution of an antibiotic solution of your dilution. You will also have M9 + glucose stock in lab. You final solution should have the prescribed antibiotic concentration and be 0.5% glucose in M9 media.

Table 3: Antibiotic concentrations

Group	**Team**	**ABX concentration (µg/mL)**	**ABX concentration (µM)**
A	1	0.1	0.21
B	1	0.21	0.44
A	2	0.43	0.90
B	2	0.90	1.88
A	3	1.87	3.90
B	3	3.90	8.13
A	4	8.11	16.90
B	4	16.88	35.17
A	5	35.11	73.15
B	5	73.05	152.19
A	6	100	208.33
B	6	150	312.50

The plate layout is shown in Figure 4. Each team will work with one concentration of the antibiotic. Five wells will be filled with the media without any antibiotic in order to measure the growth rate λ_0. Fill each of these wells with 300 µL of media.

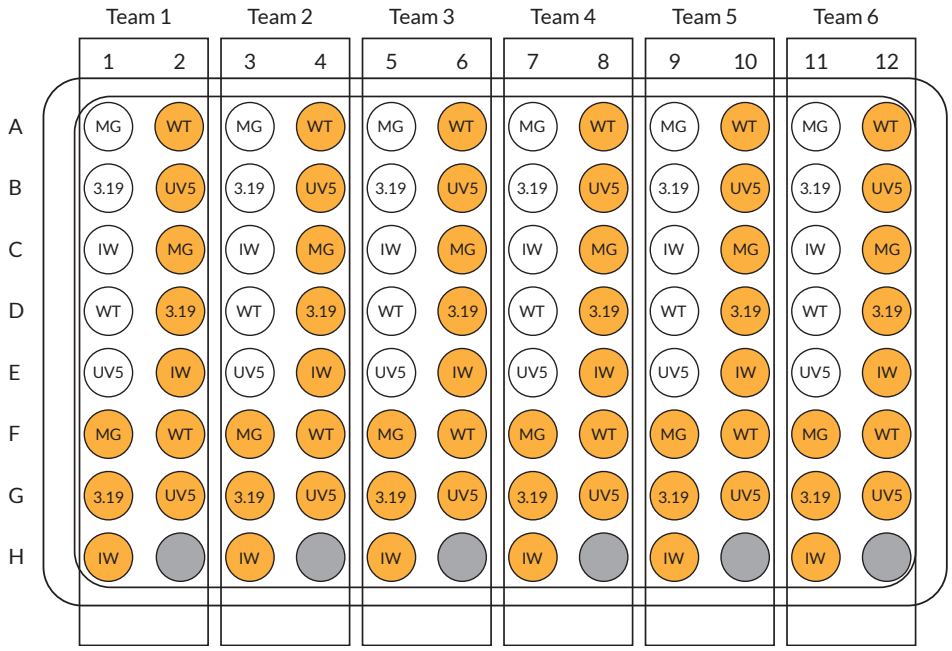

Figure 4: Plate layout for growth with antibiotic. White wells are media without antibiotic, i.e., M9 minimal media with 0.5% Glucose. Orange wells are for media with antibiotic. The gray well has media but no cells, left as a blank.

4 Assignment

Problem 0 (Summary)**.**
Write a summary of this experiment, its objectives, and conclusions between one paragraph and one page in length.

Problem 1 (Preparing the culture (prelab))**.**
Why is the culture grown for about 4 hours before the experiment? Why is it not just taken directly from the overnight culture?

Problem 2 (Drug concentrations (in-lab prelab))**.**
You will be assigned a team number when you come to lab. Work out the dilution protocol you will use to obtain a 0.5% glucose in M9 solution that has the concentration of tetracycline prescribed in Table 3.

Problem 3 (Plotting growth curves and obtaining growth rates)**.**
Plot the growth curves (OD600 measurements as a function of time). Make sure to subtract the blank measurements from your OD measurements. Find the section where cells grew exponentially and perform a fit to obtain the growth rate for each of the growth curves you have obtained.

Problem 4 (Plotting growth rates and finding $q_{\text{ex},0}^{\max}$)**.**
For each strain, make a plot with the antibiotic concentration on the horizontal axis and the relative growth rate on the vertical axis. The relative growth rate is the growth rate of the cells at a nonzero antibiotic concentration compared to growth in the absence of antibiotic. We have been describing this as λ / λ_0. Both axes should be on a logarithmic scale (though this precludes plotting points where the growth rate is identically zero).

From these curves, perform a regression to obtain estimates for $q_{\text{ex},0}^{\max}$ for each strain.

Problem 5 (RNAP binding probability)**.**
Plot your estimates for $q_{\text{ex},0}^{\max}$ versus the probability of polymerase binding, p_{bound} as given by equation (43). You can take P and N to be their typical values of $P \approx 5000$ and $N \approx 4.6$ million. Does the relationship in equation (43) appear to hold?

Problem 6 (Data and code)**.**
Attach all code used for data analysis.

References

[1] Nathan M Belliveau, Griffin Chure, Christina L Hueschen, Hernan G Garcia, Jane Kondev, Daniel S Fisher, Julie A Theriot, and Rob Phillips. Fundamental limits on the rate of bacterial growth and their influence on proteomic composition. *Cell Systems*, 12(9):924–944, 2021.

[2] Robert C Brewster, Daniel L Jones, and Rob Phillips. Tuning promoter strength through rna polymerase binding site design in *Escherichia coli*. *PLoS computational biology*, 8(12):e1002811, 2012.

[3] Ian Chopra and Marilyn Roberts. Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. *Microbiology and molecular biology reviews*, 65(2):232–260, 2001.

[4] J. Barrett Deris, Minsu Kim, Zhongge Zhang, Hiroyuki Okano, Rutger Hermsen, Alexander Groisman, and Terence Hwa. The Innate Growth Bistability and Fitness Landscapes of Antibiotic-Resistant Bacteria. *Science (80-.).*, 342(6162):1237435–1237435, nov 2013.

[5] Matthew Scott, Carl W Gunderson, Eduard M Mateescu, Zhongge Zhang, and Terence Hwa. Interdependence of Cell Growth and Gene Expression: Origins and Consequences. *Science (80-.).*, 330(6007):1099–1102, nov 2010.