Math 485

Modeling Multistability in the Expression of the *lac* Operon in *Escherichia coli*

Analysis of:
"Multistability in the lactose utilization network of Escherichia coli"

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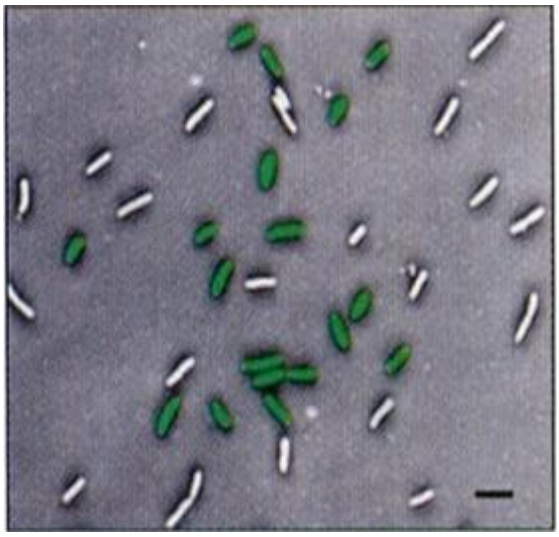

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Abstract

The *lac* operon is a segment of DNA in the bacteria *Escherichia coli* that controls the metabolism of lactose in the absence of glucose, its preferred carbon source. The expression of this gene segment is highly regulated in order to maximize efficiency and minimize energy waste within the cell. Due to the presence of a positive feedback loop within this system, the mathematical model of the lactose utilization network is multistable. For our project, we recreated and verified the mathematical model presented by Ozbudak et al. in their paper, "Multistability in the lactose utilization network of *Escherichia coli*." We also thoroughly analyzed the dynamics of the system by breaking down the model and varying its parameters, linearizing and classifying its fixed points, and creating a simulation that generates data points based on fixed initial conditions. As a result of this analysis, we were able to quantitatively characterize the behavior of the expression of the *lac* operon.

Introduction

The multistability of a system contributes to the existence of a phenomenon called biological switching. A multistable system has the capacity to develop many internal states from only one set of external inputs; the potential for this multistability is the defining characteristic of a switch. Biological switches play a role in a variety of systems within cells - including whether a cell multiplies through the process of mitosis - and thus studying the underlying mathematics is an important step in understanding (and predicting) an organism's fate.

Gene expression is another process within a cell that can be described by analyzing the multistability of the system. In the bacteria *Escherichia coli*, a segment of DNA called the *lac* operon is responsible for metabolizing lactose. This gene is not expressed consistently; rather, it is turned on only when there is an absence of glucose, and the metabolism of lactose is crucial to keep the cell alive. There exists a positive feedback loop in the regulatory network of this system, which creates the potential for multistability. The

resulting multistability is responsible for turning the expression of this gene “on” and “off” at any given time - that is, it is responsible for flipping the biological switch.

In their paper, Ozbudak et al. performs experiments that measure the expression of the *lac* operon in a variety of situations. This allows them to develop a mathematical model that describes the behavior of the system, and this analysis results in the generation of a phase diagram. The phase diagram describes the internal states of the system (whether the *lac* operon is expressed) as external parameters are varied (glucose and lactose). This presents the criteria that determines how to produce a functional biological switch.

Our group has reconstructed and verified the results of the paper by Ozbudak et al. and analyzed their mathematical model. We performed parameter reduction, system linearization, and fixed point analysis to determine the behavior of the system as parameters are varied. We also generated our own data points in the confines of the initial conditions of the system to determine if our reconstructed simulation produced an accurate representation of the system. This data was compared to that of Ozbudak et al. We then interpreted the mathematical analysis in terms of the biological consequences.

Background

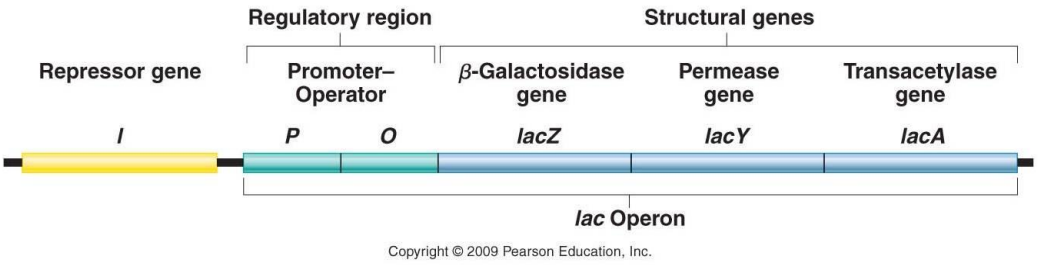
Biological Background:

Escherichia coli is a type of bacteria that is found in the intestines of various mammals. *E. coli* is considered a model organism because it is (relatively) harmless, easy to breed, and contains genes similar to those of humans. As a result, it is one of the most common types of cells used in biological research. In their paper, Ozbudak et al. studied a segment of *E. coli*’s DNA called the *lac* operon. DNA is like a recipe book found in every living cell. This book has all of the instructions required to make functional proteins. The process of protein production includes transcription (reading the recipe) and translation (making the protein from the recipe). The *lac* operon in *E. coli* is a gene segment that specifically codes for proteins that are responsible for breaking down lactose.

E. coli prefers to obtain and break down - that is, metabolize - glucose in order to gain carbon molecules. These carbon molecules are used to complete various functions in the cell. The uptake and breakdown of glucose is the most energy-efficient way to obtain these carbon molecules. However, when there is not enough glucose in the cell, *E. coli* will metabolize lactose instead. When lactose is present, the *lac* operon comes into play; *E. coli* essentially "turns on" the *lac* operon, and it begins creating proteins and enzymes that are built to break down lactose. This is called *lac* operon expression - though it is usually turned off, when lactose is present, the gene segment is expressed (turns on), and is transcribed and translated. When this happens, lactose is broken down into two smaller sugars: glucose and galactose. These sugars are then used for their carbon molecules in a variety of cell processes.

So, how is the *lac* operon turned on?

An operon is multiple genes in a sequence. Below is a simple diagram of the organization of gene segments on the *lac* operon:



FFigure (1)

The first three genes, *I*, *P*, and *O*, make up the controlling region, and determine whether the *lac* operon is on or off at any given moment. The last three genes, *lacZ*, *lacY*, and *lacA*, are the structural genes. They code for the proteins and enzymes that break down lactose.

The *P* segment is the promoter region, which functions as an attachment site for RNA polymerase, an enzyme that reads the DNA segment. Next to the promoter is the operator

(*O*) region on the DNA. This site is where the repressor protein for the *lac* operon attaches. The repressor protein is called LacI (I=inhibitor). When glucose is present, LacI is bound to the operator, preventing RNA polymerase from reading the gene segment. This stops expression of the *lac* operon (keeps it turned off). When there is lactose present in the cell, it binds to the repressor, which lifts LacI off of the operator. RNA polymerase is then able to transcribe the *lac* operon, turning on its expression, and lactose is metabolized.

Figure (2) illustrates the portions of the gene segment that function as attachment sites for proteins.

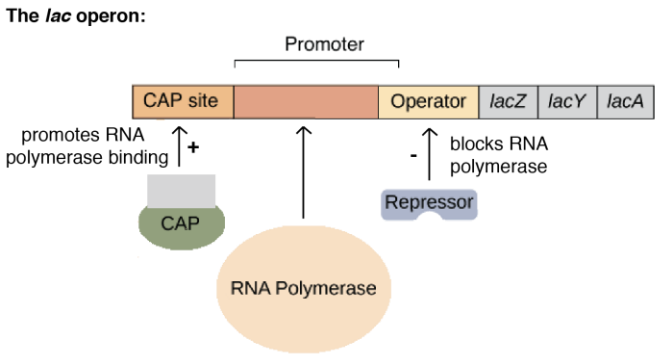


Figure (2)

There is also a second level to *lac* operon control and expression. This two-pronged control system prevents the system from metabolizing lactose if there is *any* glucose present in the cell; this ensures that the cell does not waste energy breaking down lactose if the preferred carbon source - glucose - is available. At the promoter region at the beginning of the operon, RNA polymerase binds, along with a protein called the catabolite activator protein (CAP). For the *lac* operon to be turned on, the CAP must be bound to a molecule called cyclic AMP (cAMP). cAMP is essentially a direct measure of the level of glucose in the cell. When glucose levels are high, there is low cAMP in the cell, and when glucose levels are low, there is high cAMP in the cell. So, if glucose is present, the CAP-cAMP complex does not bind efficiently, and the *lac* operon does not turn on.

The table below summarizes *lac* operon expression in a variety of situations.

Situation	**Result**	***Lac* Operon Expression**
High glucose, no lactose	Repressor protein bound to operator; low cAMP; RNA polymerase activity blocked	None
Glucose AND lactose	Lactose bound to repressor protein (released from operator); low cAMP; RNA polymerase cannot efficiently bind	Some; inefficient
No glucose, high lactose	Lactose bound to repressor protein (released from operator); high cAMP; RNA polymerase active	High
No glucose, no lactose	Repressor protein bound to operator; high cAMP; RNA polymerase activity blocked	None

Table (1)

The next segments on the *lac* operon are *lacZ*, *lacY*, and *lacA*. (Please note that gene segments are always italicized. This is an important distinction, as the products the genes code for may go by the same name; the protein products will not be italicized.) These genes are all recipes for enzymes that facilitate the metabolism of lactose. *LacZ* codes for β-galactosidase, the enzyme that breaks down lactose into glucose and galactose. *LacA* codes for acetyltransferase, an enzyme that facilitates these processes. *LacY* codes for lactose permease (also known as LacY, with no italics), which is a protein that helps bring more lactose into the cell. This is an important enzyme in this process because it creates a positive feedback loop. When there is lactose present in the cell, the *lac* operon is on, and thus more LacY is created. LacY brings more lactose into the cell, which must be broken down, and therefore encourages continued expression of the *lac* operon.

The positive feedback loop described above is essential to the backbone of this experiment, because it creates the potential for multistability; more specifically, the lactose utilization network of *E. coli* expresses bistability. However, it is important to mention that the validity of this system depends on having cells with well-defined initial states, as the bistable region has hysteretic behavior. Each cell must have been either never induced (the *lac* operon has

never been expressed), or fully induced (the *lac* operon is currently being expressed), as the system response is dependent upon its history.

Mathematical Background:

Modeling Positive Feedback and Bistability

In order to model the bistability of the *lac* operon, three equations are used. The first equation models the relationship between the concentration of LacI (the repressor protein) and the intracellular concentration of TMG. This equation denotes the active fraction of LacI in the system.

$$\frac{R}{R_T} = \frac{1}{1+(x/x_0)^n}$$

Equation (1)
Ozbudak et al.

R is the concentration of active LacI, R_T is the total concentration of LacI, *x* is the intracellular concentration of TMG, x_0 is the half-saturation of TMG, and the exponent, *n,* is the Hill coefficient. For modeling purposes x_0 can be chosen and is set to 1, and the hill coefficient *n* is set to 2 based off of experimental evidence.

Equation (1) behaves as a decreasing sigmoidal function of *x*. This is the case because even the smallest amount of binding of TMG to LacI will interfere with its inhibitory activity, and as more TMG binds, the level of inhibition of the *lac* operon increases.

The second equation gives the rate of generation of lactose permease (LacY). Recall that as TMG binds, LacY is expressed and facilitates the uptake of more TMG. This makes up the positive feedback loop. *Equation (3)* shows that the generation of LacY is a decreasing hyperbolic function of LacI.

$$\tau_y \frac{dy}{dt} = \alpha \frac{1}{1 + R/R_0} - y$$

Equation (2)

Ozbudak et al.

In *Equation (2)*, *y* is the concentration of LacY, τ_y is a time constant, α is the maximum value of growth of LacY. The minimal value achieved is α/ρ, where $\rho = 1 + R_T/R_0$, which is the repression factor. The repression factor describes how well LacI can regulate expression of the *lac* operon.

The third equation gives us the rate of change of the intracellular concentration TMG.

$$\tau_x \frac{dx}{dt} = \beta y - x$$

Equation (3)
Ozbudak et al.

Here, β is the measure of TMG uptake per LacY molecule. TMG enters the cell at a rate proportional to the concentration of LacY in the cell, and it is diminished in a first order reaction with time constant τ_x.

Equations (1,2,3,4) may be combined to retrieve the steady state result:

$$y = \alpha \frac{1 + (\beta y)^2}{\rho + (\beta y)^2}$$

Equation (4)
Ozbudak et al.

ρ, α, and β are functions of concentrations of glucose (G) and TMG (T), the system inputs. As these three arbitrary parameters are varied, the number and stability of the fixed points change in nature. Varying parameters results in saddle node bifurcations.

Equation (4) can be rewritten as a cubic equation, as follows:

$$y^3 - \alpha y^2 + (\rho/\beta^2)y - (\alpha/\beta^2) = 0$$

Equation (5)
Ozbudak et al.

To attack this, it should be recalled how to deal with general cubic equations with two identical roots. We choose to deal with two roots because we seek two stable states. The general cubic can be written in the following form:

$$(y-a)(y-a)(y-\theta a) = \\ y^3-(2+\theta)ay^2+(1+2\theta)a^2y-\theta a^3$$

In the above equation, θ is the dimensionless ratio of roots. By rewriting *Equation (5)* in this form and comparing coefficients, we can define our arbitrary functions.

$$\rho = (1+2\theta)(1+2/\theta)$$
$$\alpha\beta = (2+\theta)^{3/2}/\theta^{1/2}$$

Equations (6) and (7)
Ozbudak et al.

Equations (6, 7) denote the boundary of the bistable region.

Measuring Network Parameters

As mentioned earlier, ρ, α, and β are functions of inputs of glucose (G) and TMG (T). These parameters are functions of the initial conditions (G and T), and these functions are determined by experimental data. Some information on *how* they were derived by Ozbudak et al. will be provided.

The physical meanings of the network parameters:

- α refers to the level of *lac* operon expression that would be seen if every repressor molecule were inactivated. α is the maximum induction level.
- ρ refers to the ratio of the maximal induction level to the basal induction level. The basal induction level is the level of *lac* operon expression that would be seen if every repressor molecule were activated. ρ is the repression factor.
- β refers to the rate of TMG uptake per molecule of LacY.

Calculating the functional equations of these three parameters required numerous single-cell experiments, where their dependence on glucose and TMG levels were measured. See *Figure (3)* for the results of these *in vivo* experiments.

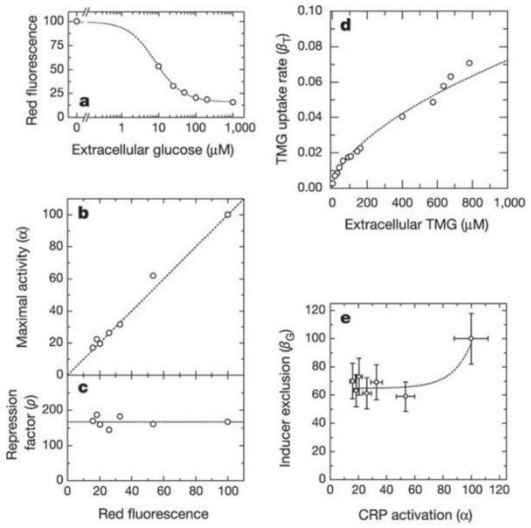


Figure (3)

These calculations also required the application of some caveats. First, the saddle node condition was applied at each boundary of the bistable region; this was done separately at what was referred to as ON (induced) and OFF (uninduced) regions. Second, it was taken into account that α was approximately 15% higher at the OFF threshold than the ON threshold. Third, due to low fluorescence values in the OFF region, there was a large error in the calculation of ρ. Therefore, the authors decided to estimate both α and ρ at the ON threshold only. Lastly, the net TMG uptake rate was decomposed as:

$$\beta(T,G) = \beta_T(T)\beta_G(G)$$

Recall that *T* refers to TMG concentrations and *G* refers to glucose concentrations. The power law was assumed for the first half of the equation, and the least-square fitting technique was used to extract the necessary functions.

It was determined that:

$$\alpha = \frac{84.4}{1 + (G/8.1)^{1.2}} + 16.1, \rho = 167.1$$
$$\beta_T = (1.23 * 10^{-3})T^{0.6}, \beta_G(G > 10) \cong 65$$

Equations (8) and (9)
Ozbudak et al.

Methodology

Biological Methods:

To explore the multistability of the lactose utilization network, Ozbudak et al. measured the expression of the *lac* operon in *E. coli* cells in a variety of conditions. This required a method that would allow for the visualization of operon expression; two reporter proteins were inserted into the cell's DNA to measure relative levels of expression. Ozbudak et al. inserted a copy of the green fluorescent protein (GFP) into the cell's DNA at the site of *lac* promoter, and red fluorescent reporter (HcRed) at the site of the *gat* promoter. GFP served as a direct indicator of *lac* operon expression, and HcRed essentially revealed the levels of the CAP-cAMP complex.

Using the reporter proteins as indicators, the behavior of cell populations was measured for *lac* operon expression as levels of extracellular glucose and lactose were varied. Since the presence of GFP indicates *lac* expression, observing at what concentrations of lactose GFP is present will determine when the *lac* operon is multistable. A population of 1000 *E. coli* cells were observed in varying levels of lactose. At certain levels of lactose, the *lac* operon is either expressed or not. In a particular range of lactose, the *lac* operon is multistable. In this range of lactose, some of the 1000 cells will be induced and will produce GFP and some will not. In this range, the expression of the *lac* operon is multistable because it can either be induced or not.

It is important to note that in this particular experiment, levels of thiomethylgalactoside (TMG) were measured instead of lactose. TMG is a lactose analog that cannot be readily metabolized by the system.

It is also important to note that the cells used in this experiment had well-defined initial states. More specifically, each cell was either uninduced (the *lac* operon had never been expressed), or fully induced (the *lac* operon was currently being expressed).

Mathematical Methods:

3D Manifold

To analyze the steady state solution of the system (*Equation (4)*) a change in variables was performed to reduce the total number of parameters from 2 to 3. The change in variables goes as follows:

$$y = \alpha z$$

Equation (10)

Following substitution of the newly z:

$$z = \frac{\frac{1}{\rho^2} + (\frac{\alpha\beta}{\rho} z)^2}{\frac{1}{\rho} + (\frac{\alpha\beta}{\rho} z)^2}$$

Equation (11)

Variables are then redefined as follows:

$$\mu = \frac{1}{\rho}$$

Equation (12)

$$\lambda = \frac{\alpha\beta}{\rho}$$

Equation (12)

The steady state equation of the dynamical system with variable changes now looks as follows:

$$z = \frac{\mu^2 + (\lambda z)^2}{\mu + (\lambda z)^2}$$

Equation (13)

Using *Equation (13)*, the steady state solution can now be plotted in three dimensions to visualize the bistable region, where the folding occurs, and both induced and uninduced monostable regions. In further analysis *ρ* and *α(G)* will be held constant and a specific cut of the manifold with varied *β(T)* will be analyzed. See the *Figure (4)* below:

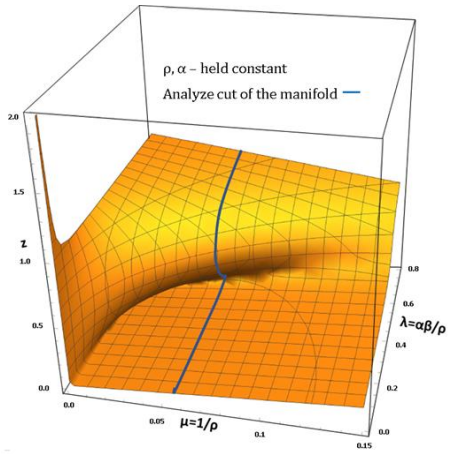


Figure (4)

Fixed Points Analysis

Provided *Equations (1,2,3),* the vector field on the line for y can be analyzed. Time constants are ignored and the derivative for *Equation (3)* is set to 0 to analyze the fixed points.

$$x = \beta y$$

Equation (14)

The equation for x is then substituted into *Equation (2),* yielding the following equation:

$$\frac{dy}{dt} = \frac{\alpha}{1 + \frac{\rho - 1}{a + (\beta y)^2}} - y$$

Equation (15)

After simplification:

$$\frac{dy}{dt} = \frac{\alpha\,(1+x^2)}{(\rho+x^2)} - y$$

Equation (16)

Setting α = 3, and β = 5, the Vector field on the line can be plotted. Note that function appears to be a cubic and that the derivative of y intersects y = 0 three times for the given parameter set. This indicates that there are at most three fixed points. See *Figure (5)*.

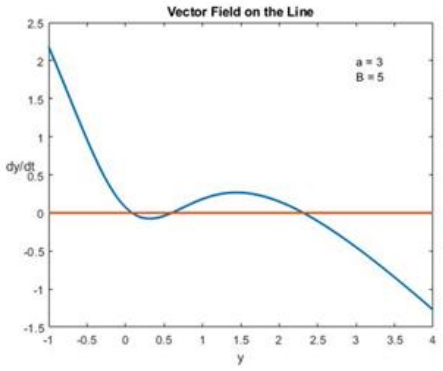


Figure (5)

A linearization of the fixed points is performed to classify fixed point type. To do this, the Jacobian is computed.

$$\mathrm{J(x,y)} = \begin{pmatrix} \partial_x(\beta y - x) & \partial_y(\beta y - x) \\ \partial_x(\frac{\alpha(1+x^2)}{\rho+x^2} - y) & \partial_y(\frac{\alpha(1+x^2)}{\rho+x^2} - y) \end{pmatrix}$$

Equation (17)

From the Jacobian, the trace and the determinant are computed

$$\Delta = 1 - \frac{2\alpha(\rho-1)\beta^2 y}{(\rho+(\beta y)^2)^2}$$

Equation (18)

$$\tau = -2$$

Equation (19)

The trace is a negative constant and the determinant is only dependent on *β(T)*, recalling that α and ρ are held constant, we can conclude that the fixed points will either be saddles or stable nodes. See *Figure (6)* below for visualization.

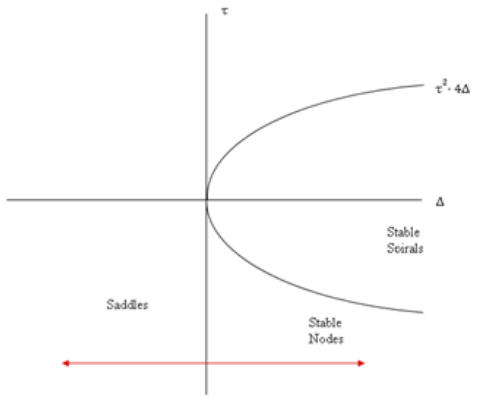


Figure (6)

To confirm the findings from the linearization the vector field on the line is plotted along with the corresponding vector field using PPlane software. For *Figures (7, 8),* α = 100 and β = 0.1864. The red circle in the plots indicates the location of a stable node. This fixed point represents the "off" state of the lac operon.

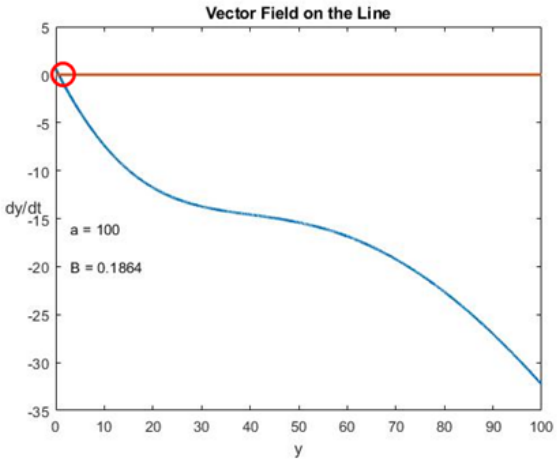


Figure (7)

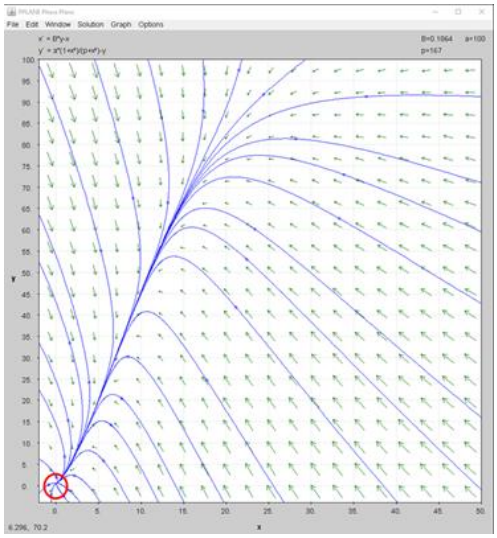


Figure (8)

To analyze the other relevant fixed points, β is varied. *Figures (9, 10)* represent the vector field on the line and the corresponding vector field for α = 100 and β = 0.489. The red circles in the plots indicate the location of a stable node and the black circle indicates the location of a saddle. These figures display bistability under the given parameter set, with the first stable node showing the "off" state and the second stable node showing the "on" state of the *lac* operon.

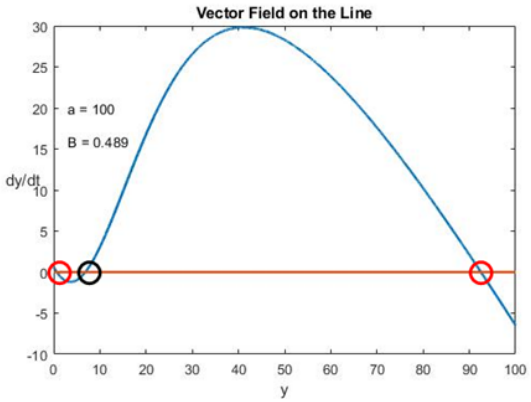


Figure (9)

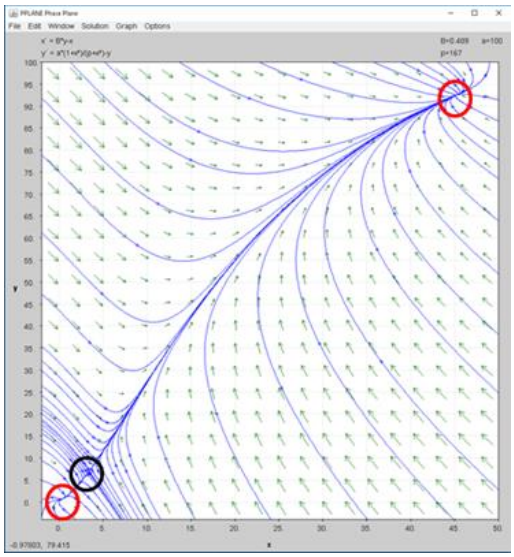
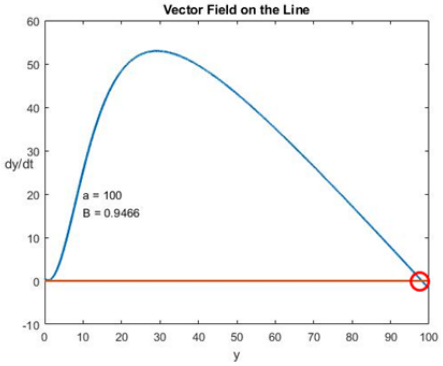


Figure (10)

Again, β is varied to look at the last relevant state of the system. *Figures (11, 12)* represent the vector field on the line and the corresponding vector field for α = 100 and β = 0.9466. The red circle indicates the location of a stable node. This stable node represents the "on" state of the *lac* operon.



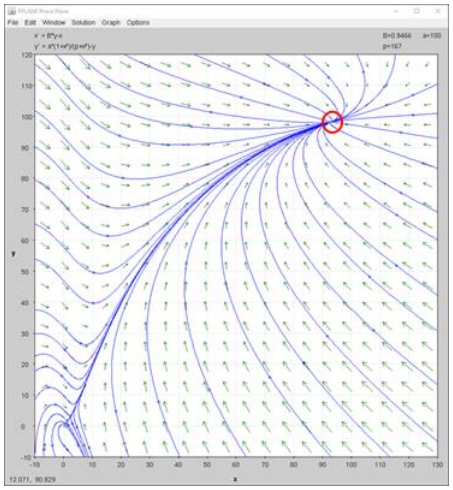


Figure (11)

Figure (12)

Sigmoidal Switching

To analyze the switching behavior of the dynamical system, the ordinary differential equations from *Equations (2,3)* were simulated in MATLAB using function ODE45. Parameters were selected so $\alpha = 3$ and $\beta = 6.2$. This set of parameters allows the turning on of the *lac* operon to be visualized. Paying attention to the blue line in *Figure (13)*, we see that *y* goes from a value of 2 to 16 and looks similar to a step function indicating that lactose breakdown will be initialized given the proper parameter values.

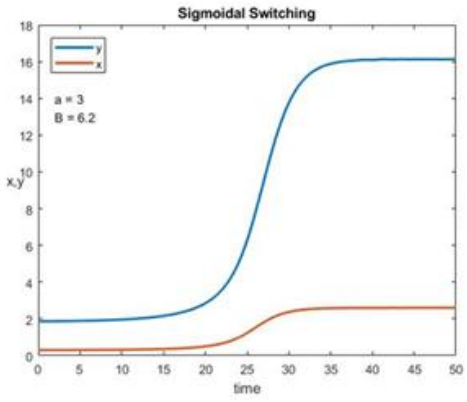


Figure (13)

Simulation

Experimental data from Ozbudak et al. will be used as a reference in comparison to the MATLAB simulations that were performed. See *Figure (14)* for reference. Note that the colored circles, green, blue, purple, and red have corresponding matches on in following phase diagrams pictured in *Figures (15, 16, 17)*. The bottom panel displays the uninduced state of the *lac* operon and the top panel displays the induced state of the *lac* operon.

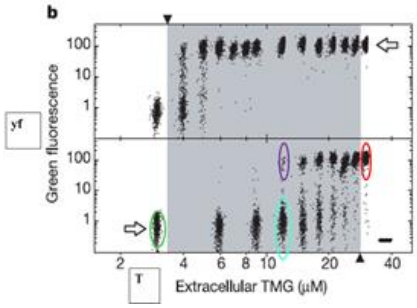
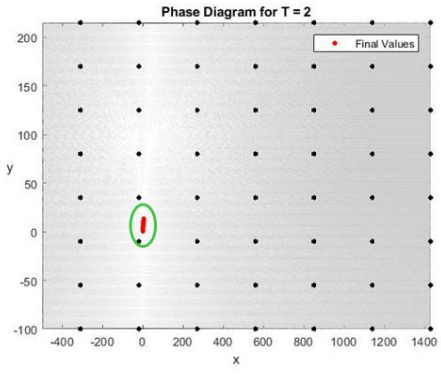


Figure (14)

Using ODE45 in MATLAB, a range of initial conditions x_0 and y_0 were selected to encapsulate all relevant fixed point values. The array of initial conditions can be visualized in as black dots in *Figures (15, 16, 17)*. Additionally, the vector fields were added to the background of all off the phase diagrams for these plots. A darker grey hue shows that initial conditions will flow more quickly whereas the light grey hue shows initial conditions will travel more slowly as they approach their final values. The final y_f values are expected to be found in the region of the lightest colored hue. *Figure (15)* shows the dynamical simulation for T = 2, *Figure (16)* shows the dynamical simulation for T = 10, and *Figure (17)* shows a dynamical simulation for T = 30. The simulation ran for t = 10s in order to avoid complete convergence to y_f steady state values.



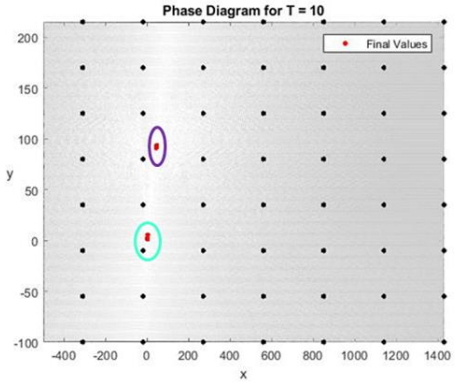


Figure (15) *Figure (16)*

Figure (17)

After the simulation was run with parameter values of greatest interest, it was reran with a range of T values from 1 to 40. Simulated y_f vs. T plots were constructed as seen in *Figure (18)* and then compared to experimental data from *Figure (14)*. For supplemental simulation videos, please visit the Appendix.

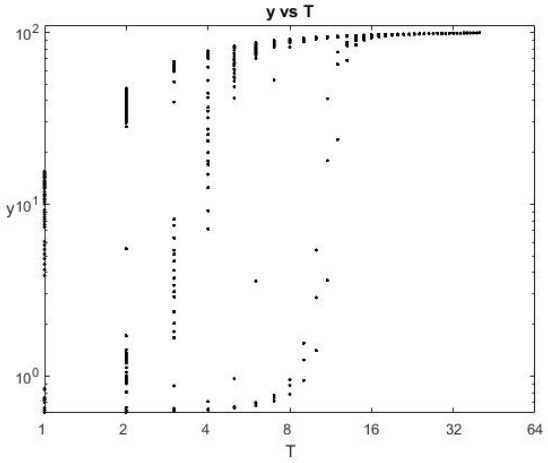


Figure (18)

Qualitative Comparison to Ozbudak et al.'s Data

Unfortunately, the data gathered from our system did not equate with values calculated by Ozbudak and the other authors of the paper or from experimental data. Therefore, a

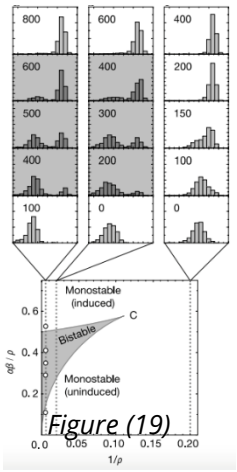


Figure (19)

qualitative comparison between the data from our system and Ozbudak's data will have to suffice instead of a quantitative comparison. To do this, it is important to take note of the following graph again and compare Ozbudak's data distributions to our own.

In *Figure (19)*, the three dotted vertical lines correspond to differing values of $1/\rho$ that "cut" the manifold discussed earlier. The two vertical lines on the left are situated in such a way that as the extracellular TMG concentration is increased (which in turn increases βsince it is an increasing function of TMG), the system switches from monostable to bistable and back to monostable. On the other hand, the dotted vertical line on the right illustrates how as the extracellular TMG concentration increases, the system moves from the lower stable point to the higher stable point in a graded fashion (it never switches to bistable. The three series of panels above each of these three dotted lines illustrate the distributions of the natural log of the green fluorescence values as the TMG level varies (the TMG level is the number in the box in each panel).

Compare this to the distribution of the log of the green fluorescence values from our simulation:

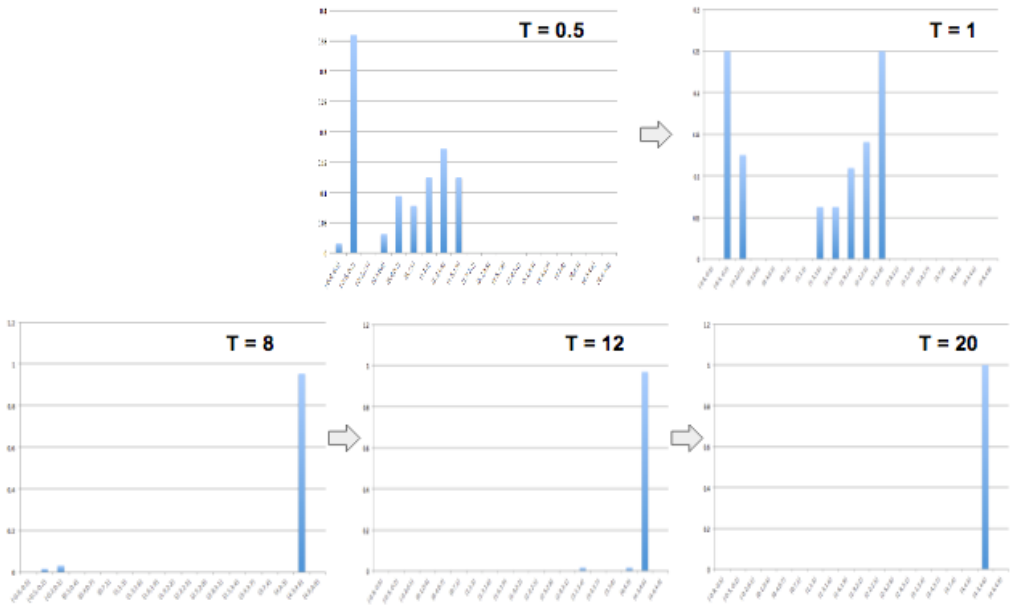


Figure (20)

These are distributions of the natural log of green fluorescence values generated by our system after ten seconds for differing levels of extracellular TMG concentrations (which are labeled on each graph). The distributions generated by our system can be fairly closely related to one of the series of distributions on the previous figure where data from Ozbudak et al. data moves from monostable to bistable and back to monostable. It is clear that in our series of distributions, as extracellular TMG concentrations increase, the congregation of green fluorescence values shifts from the lower stable point to the higher stable point.

One stark contrast between our distributions and Ozbudak's is that for low levels of extracellular TMG, the green fluorescence distribution is still bistable when it should be closer to monostable. This difference is most likely due to an error in the setting of the initial conditions. Additionally, it is clear that as higher TMG values are approached, nearly all of the green fluorescence values converge to the higher stable value, which is very reminiscent of Ozbudak's distributions as well as actual experimental data. However, it should be noted that Ozbudak's distributions exhibit much more variability than the distributions our system generated. More variability is more reminiscent of experimental data.

Results

Biological Results:

The results of the biological experiment performed by Ozbudak et al. is included in *Figure (21)*. This figure shows that induction of the *lac* operon takes place hysteretically (see the grey bistable region that cuts through the graph). The cells increase and decrease their expression of the *lac* operon discontinuously as they cross the boundaries of the bistable region (the so-called "switching thresholds"). In other words, initially uninduced cells are only turned on if the TMG concentration is *above* a certain level, and initially induced cells are only turned off if the TMG concentration is *below* a certain level. This tells us that the response of the system to external conditions is dependent upon the system's history.

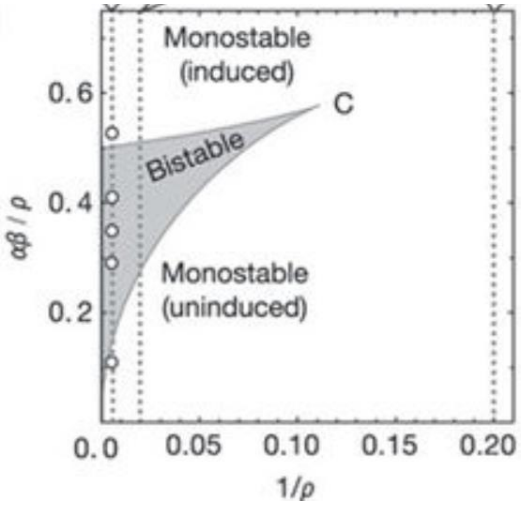


Figure (21)

The experiment also successfully determined the approximate TMG levels at which these biological "switches" occur. When there is no glucose present in the cell, *lac* operon expression is turned off at TMG concentrations below 3μM and turned on at TMG levels above 30μM. However, when the system is between these switching thresholds, its response is fully dependent on the history of the cell. TMG levels must drop below 3μM to turn off initially induced ("on") cells and must exceed 30μM to turn on initially uninduced ("off") cells. The bistable region occurs between 3μM and 30μM. In this region, some of the 1000 *E. coli* cells that were observed were induced and some were not. Initially, in the bistable region, more cells are uninduced than not. As the concentration of TMG increases, more cells are induced and produce GFP until the concentration reaches 30μM. After which, the *lac* operon is fully induced.

Mathematical Results:

Ozbudak et al. generated a phase diagram based on their mathematical model of the lactose utilization network of *E. coli*. This phase diagram shows us that cells may shift from

being uninduced to induced, or vice versa, hysteretically or in a graded fashion. In the bistable region, the system is hysteretic and behaves in the same way as the wild-type system. However, this region of bistability decreases as the repression factor (degree of *lac* operon expression), ρ, decreases. At a critical factor of ρ=9, the bistable region hits a cusp and comes to an end, and the system response occurs in a graded fashion. That is, expression of the *lac* operon in each cell can move continuously between low and high values. This can be seen in the white regions of the graph.

Analysis of fixed points indicates that increasing the amount of extracellular TMG, parameter T, causes the *lac* operon to switch from the "off" position to the "on" position. Expected switching behavior was confirmed using MATLAB ODE45 simulations as depicted in *Figure (13).* After running larger scale simulations with a range of initial conditions it can be concluded that the dynamical system representing the lactose utilization network is accurate displaying sigmoidal switching behavior. The model was not able to properly replicate induced and uninduced states as displayed in *Figure (14)*, this means that the simulation did not capture the effect of hysteresis.

Discussion & Conclusion

The mathematical analysis above provides a quantitative representation of how *lac* operon expression behaves as the amounts of lactose and glucose in the cell are varied. There are three critical states of this system: two states with single stable fixed points, and one state with two stable fixed points separated by a saddle node. Each of these mathematical states represent different states in the cell; we will go through each state separately.

In *Figure (8)*, we see that there is one stable node. This node corresponds to the *lac* operon being turned off. At these given values of lactose and glucose (low lactose and high glucose), the *lac* operon is never being expressed. The initial conditions of the cell may be varied, but the external parameters of the system dictate that the *lac* operon is not being

expressed. This saves energy in the cell, as it is not wasted producing enzymes that are not necessary at this given time.

Conversely, in *Figure (12)*, we see another stable node in a different position. This node corresponds to the *lac* operon being turned on. When lactose is abundant in the cell, the *lac* operon is always being expressed in order to maximize lactose metabolism. In this case, despite any varying of initial conditions in the cell, *lac* operon expression is always turned on. This ensures that the cell is able to maintain its normal functions by using lactose as an energy source, since there is no glucose available.

The most biologically interesting case is that shown in *Figure (10)*. In this case, there are two stable nodes that are separated by a saddle. In this case, the cell's response to the system is hysteretic, and the *lac* operon may or may not be expressed. Depending on the initial conditions of the cell, it may move towards either one of the fixed points; this means that the *lac* operon will not be expressed if it moves to the lower fixed point, but will be expressed if it moves towards the higher fixed point. The cell's initial state also plays a large role in the fate of these particular cells. If the *lac* operon was previously uninduced in the cell, it will take larger amounts of lactose to turn on its expression; similarly, if the operon was previously induced, it will take lower amount of lactose to turn its expression off. It is also important to note that one single cell cannot exist in both states - the bistability exists in a large population of cells with the same initial conditions, some of which respond by turning the *lac* operon on, and others keeping it off. There is a level of stochasticity that contributes to this "decision," but this has not been taken into account in this particular model.

Though it is fairly easy to visualize the effects of hysteresis on this system by analyzing the phase diagrams, the MATLAB simulations were unable to display the effects of hysteresis. This occurred due to the selection of initial conditions x_0 and y_0, which were originally chosen to cover all possible fixed point values. To improve the current model, initial conditions could be selected to correspond with induced and uninduced states. Ideally,

initial conditions would be selected based on the original experimental data, which was not available during the simulation trials.

Appendix

https://www.youtube.com/watch?v=V-MgNAJtNJw&feature=youtu.be

https://www.youtube.com/watch?v=kL_k8FZYmoQ&feature=youtu.be

https://www.youtube.com/watch?v=AHUsa-4BrcQ&feature=youtu.be

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