

# Gene Regulatory Networks

Hal L. Smith\*

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## 1 The Tryptophan operon model

Tryptophan is one of the 20 amino acids that link together to form proteins. For humans it is an essential amino acid, we must get it from our diet since we cannot synthesize it. *E. coli* bacterial cells, however, can synthesize the amino acid tryptophan when it is not provided from the environment (e.g. the gut where it lives). It would be wasteful to synthesize tryptophan when it is readily available in the environment so *E. coli* have evolved a genetic switch (the trp operon) that turns off synthesis in this case and turns on synthesis when tryptophan is no longer present in the environment. The following model is based loosely on the control of tryptophan production by the trp operon. Although the trp gene codes for 5 enzymes that act on five substrates, or precursors, we simplify it a bit by considering only one of each. Our treatment follows Banks and Mahaffy [2], who considered a general negative feedback genetic control model. Their model, hence ours too, does not include transcriptional attenuation of the trp operon. See Santillan and Mackey [11] for a more realistic model of the trp operon. Our references contain further related material. Check the web page <http://science.nhmccd.edu/biol/operon/ton.html> for an animated description of the trp operon.

## 2 Reactions

Name	Symbol	Description
DNA	DNA	the gene of interest
RNA Polymerase	RNAP	enzyme that reads DNA producing mRNA
mRNA	mRNA	messenger RNA-working copy of the gene
tRNA	tRNA	transfer RNA-converts mRNA code into protein E
Enzyme	E	protein product of gene
Precursor	X	substrate converted to tryptophan by catalyst E
tryptophan	T	amino acid required by the cell
Prerepressor	P	becomes a repressor when complexed with tryptophan
Repressor	R	blocks the translation of DNA when bound to DNA

Table 1: Main Players: the chemical species we will follow in the model

Its useful to begin with a verbal model of the trp operon. It begins with RNA polymerase binding to DNA, the gene, and initiating the process of copying the DNA code to messenger RNA. This is called transcription. Messenger RNA then must be translated into protein, in our case, the enzyme E. This is facilitated by various transfer RNAs which bind a particular triplet of nucleotides and its corresponding amino acid and sequentially build the protein in the molecular machine called a ribosome. This process is called translation. The enzyme E then catalyzes a reaction whereby precursor molecule X is converted to tryptophan. The cell has now synthesized the needed protein. But now things get interesting. Two molecules of tryptophan can form a complex with a prerepressor molecule forming a repressor molecule, so-called because it can bind

\*Department of Mathematics, Arizona State University, Tempe, AZ 85287, [halsmith@asu.edu](mailto:halsmith@asu.edu)

to a site on the DNA, preventing RNA polymerase from binding there, and thus shutting down transcription of the gene and the formation of tryptophan. In this way, tryptophan controls its own synthesis. If tryptophan is readily available in the cells environment then there will be enough of it to bind with the prerepressor and shut down the cells own tryptophan synthesizing process. However, if the external supply of tryptophan is abruptly shut off, the internal concentration will decline as the cell uses up its supply of tryptophan and eventually there will be too little bound with prerepressor forming repressor so RNA polymerase is free to bind to DNA and initiate transcription which leads to tryptophan synthesis. This is a very simple switch! Now lets say this mathematically.

We now give a set of chemical reactions describing the interaction of the above mentioned molecules. In formulating equations for the concentrations of these chemical species we must acknowledge that a single cell may not contain sufficiently many molecules of a particular chemical species to justify a continuum model. For example, a typical cell will have only one copy of the gene. Therefore, to justify our model we must focus not on a single cell but rather on a collection of synchronously dividing cells, which will contain large numbers of the relevant chemical species.

**Transcription:**-RNA Polymerase reads DNA copying the DNA instructions into an mRNA "transcript":



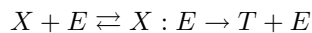
with rate constants  $k_{1+}$ ,  $k_{1-}$ ,  $k_1$ . Subscript "+" indicates forward reaction and subscript "-" indicates the backward reaction in  $\rightleftharpoons$ . RNAP:DNA denotes a complex formed when RNA Polymerase binds to DNA. It can either unbind into its constituents DNA and RNAP or proceed along the gene segment translating the DNA code to MRNA. We use this ":" notation to denote a complex hereafter.

**Translation:**-tRNA converts mRNA code into protein  $E$ :



with rate constants  $k_{2+}$ ,  $k_{2-}$ ,  $k_2$ .

Enzyme catalyzes formation of Endproduct protein  $T$  (tryptophan) using precursor  $X$ :



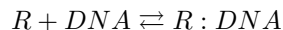
with rate constants  $k_{3+}$ ,  $k_{3-}$ ,  $k_3$ .

$n$  molecules of Endproduct combine with a Prerepressor to form Repressor  $R$ :



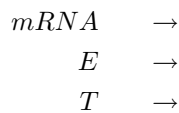
with rate constants  $k_{4+}$ ,  $k_{4-}$ . For tryptophan,  $n = 2$ .

Repressor binds to DNA making it unavailable for transcription to mRNA:



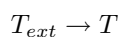
with rate constants  $k_{5+}$ ,  $k_{5-}$ .

Most molecules are degraded at some rate:



with rate constants  $d_1$ ,  $d_2$ ,  $d_3$ . Of course, they degrade to something but we will not need these. Another interpretation of these loss terms is to imagine an exponentially growing aggregate of cells in which case all chemical species suffer dilution as each cell roughly doubles in size before dividing. Thus degradation is merely dilution due to growth. Tryptophan  $T$  is also used in other cellular processes and  $d_3$  reflects this demand as well.

Finally, the exterior environment of the cell **may** provide tryptophan so we include this potential source



with rate constant  $k_6$ . Of course, the whole point of the tryptophan gene is to be able to synthesize tryptophan when  $T_{ext} = 0$ .

### 3 Assumptions

Total  $DNA$  is constant-denoted by  $DNA_T$ .

$$DNA_T = DNA + RNAP : DNA + R : DNA \quad (3.1)$$

RNAPolymerase, tRNA, PreRepressor, Precursor concentrations are nearly constant

$$\frac{d}{dt}RNAP = \frac{d}{dt}tRNA = \frac{d}{dt}P = \frac{d}{dt}X = 0$$

Since  $P$  is constant, we have:

$$0 = \frac{d}{dt}P = k4_-(R) - k4_+(P)(T^n) \quad (3.2)$$

Complexes:

$$C1 = RNAP : DNA, C2 = tRNA : mRNA, C3 = X : E, C4 = R : DNA$$

are in equilibrium:

$$\frac{d}{dt}Ci = 0, \quad i = 1, 2, 3, 4$$

This means:

$$\begin{aligned} 0 &= k1_+(RNAP)(DNA) - (k1_- + k1)RNAP : DNA \\ 0 &= k2_+(tRNA)(mRNA) - (k2_- + k2)tRNA : mRNA \\ 0 &= k3_+(X)(E) - (k3_- + k3)X : E \\ 0 &= k5_+(R)(DNA) - (k5_-)R : DNA \end{aligned}$$

Using these last equations together with (3.1) and (3.2) we find:

$$DNA_T = (DNA)\left[1 + \frac{k1_+}{k1_- + k1}(RNAP) + \frac{k5_+}{k5_-}(R)\right]$$

so

$$DNA = \frac{DNA_T}{1 + K1 \cdot RNAP + K5K4 \cdot P \cdot T^n} \quad (3.3)$$

where

$$K1 = \frac{k1_+}{k1_- + k1}, \quad Kj = \frac{kj_+}{kj_-}, \quad j = 4, 5.$$

### 4 Equations

$mRNA$  is involved in both Transcription and Translation. From these two reactions we get:

$$\begin{aligned} \frac{d}{dt}mRNA &= k1(RNAP : DNA) - k2_+(tRNA)(mRNA) + (k2 + k2_-)tRNA : mRNA - d1 \cdot mRNA \\ &= k1K1(RNAP)(DNA) - d1 \cdot mRNA \\ &= k1K1(RNAP) \cdot \frac{DNA_T}{1 + K1 \cdot RNAP + K5K4 \cdot P \cdot T^n} - d1 \cdot mRNA \end{aligned}$$

The protein  $E$  is an output of translation and catalyzes formation of  $T$ . From these two reactions we have:

$$\begin{aligned} \frac{d}{dt}E &= (k3_- + k3)(X : E) - k3_+(X)(E) + k2(tRNA : mRNA) - d2 \cdot E \\ &= k2K2(tRNA)(mRNA) - d2 \cdot E \end{aligned}$$

where  $K2$  is defined similarly as  $K1$ .

The endproduct  $T$  is used by the cell but it also combines with prerepressor to form repressor  $R$  which blocks further formation of  $T$ .  $T$  may be supplied by the exterior environment of the cell.

$$\begin{aligned}\frac{d}{dt}T &= k3(X : E) - d3(T) + nk4_-(R) - nk4_+(P)(T^n) + k6(T_{ext}) \\ &= k3K3(X)(E) - d3(T) + k6(T_{ext})\end{aligned}$$

Let's relabel  $mRNA = m$  and tidy up the equations a bit. Recall that  $RNAP, tRNA, X, P$  are constant so we may write:

$$\begin{aligned}\frac{d}{dt}m &= \frac{\beta}{\delta + \mu T^n} - d1 \cdot m \\ \frac{d}{dt}E &= \alpha_2 \cdot m - d2 \cdot E \\ \frac{d}{dt}T &= \alpha_3 \cdot E - d3 \cdot T + u\end{aligned}$$

Hopefully, the values of the newly introduced quantities are apparent. For example

$$\delta = 1 + K1 \cdot RNAP$$

Finally, it will be useful to scale out as many of the parameters as we can. Let

$$x_1 = m/m_0, \quad x_2 = E/E_0, \quad x_3 = T/T_0, \quad \tau = t/t_0 \quad (4.1)$$

where  $m_0, E_0, T_0, \tau_0$  are reference values to be chosen. Check that these can be selected so as to achieve the following

$$\begin{aligned}x_1' &= \frac{1}{1 + x_3^n} - \gamma_1 x_1 \\ x_2' &= x_1 - \gamma_2 x_2 \\ x_3' &= x_2 - \gamma_3 x_3 + u\end{aligned} \quad (4.2)$$

where  $' = \frac{d}{d\tau}$ ,  $\gamma_i > 0$ ,  $n = 1, 2, 3, \dots$  is a positive integer and  $u \geq 0$  is not the same as above.  $u = 0$  corresponds to a tryptophan-free exterior environment. Equations (4.2) are sometimes referred to as the "Goodwin Oscillator" after their inventor Brian Goodwin. See [5].

## 5 Study of Equations (4.2)

Define  $\bar{x}_3$  to be the unique positive root of

$$-\gamma_1 \gamma_2 u + \gamma_1 \gamma_2 \gamma_3 x_3 = \frac{1}{1 + x_3^n}. \quad (5.1)$$

Figure 1 depicts that  $\bar{x}_3$  is the abscissa of the unique point of intersection of the line  $y = -\gamma_1 \gamma_2 u + \gamma_1 \gamma_2 \gamma_3 x_3$  with the curve  $y = \frac{1}{1 + x_3^n}$ . Then

$$\bar{x}_2 = \gamma_3 \bar{x}_3, \quad \bar{x}_1 = \gamma_2 \bar{x}_2$$

describes the unique steady state  $\bar{x}$  of the system. Notice from Figure 1 that  $\bar{x}_3 \rightarrow \infty$  as  $u \rightarrow \infty$  and keep in mind that it depends on  $\gamma_i$  and on  $u$ .

Observe that the rate of mRNA production, a measure of the activity of the tryptophan gene, is given by  $(1 + \bar{x}_3^n)^{-1}$  at steady state. It falls to zero rapidly as  $\bar{x}_3$  increases and  $\bar{x}_3$  increases with increasing  $u$ . Thus, an external source of tryptophan effectively shuts down gene activity.

The simulations below suggest the behavior of the system. In Figure 2 we examine how tryptophan may be produced by the cell when there is no external supply of tryptophan ( $u = 0$ ). Note that first  $mRNA$ , then enzyme  $E$ , and finally tryptophan  $T$  rise and then approach the steady state  $\bar{x}$  in an oscillatory fashion. In Figure 3, we start at  $t = 0$  at this steady state but supply tryptophan  $u = 2$  when  $t > 0$  to the cell. The cell responds by immediately suppressing  $mRNA$  and enzyme  $E$  production—in other words—the gene is turned off.

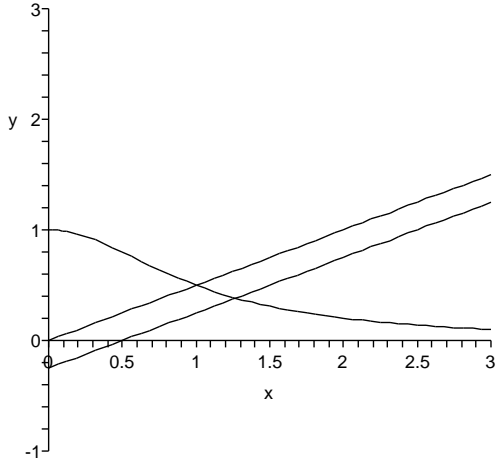


Figure 1: The  $x$ -component of the point of intersection of the two curves determines  $\bar{x}_3$  with  $n = 2$ . The case  $u = 0$  gives the top line and  $u > 0$  the lower one.

The stability of  $\bar{x}$  is determined by the linearized system

$$z' = \begin{bmatrix} -\gamma_1 & 0 & -q \\ 1 & -\gamma_2 & 0 \\ 0 & 1 & -\gamma_3 \end{bmatrix} z. \quad (5.2)$$

where

$$q = n\bar{x}_3^{n-1}(1 + \bar{x}_3^n)^{-2} \quad (5.3)$$

The characteristic polynomial is

$$(\lambda + \gamma_1)(\lambda + \gamma_2)(\lambda + \gamma_3) + q = 0$$

or equivalently

$$\lambda^3 + (\gamma_1 + \gamma_2 + \gamma_3)\lambda^2 + (\gamma_1\gamma_2 + \gamma_1\gamma_3 + \gamma_2\gamma_3)\lambda + \gamma_3\gamma_2\gamma_1 + q = 0$$

In order to simplify the algebra, we hereafter assume that

$$\gamma = \gamma_1 = \gamma_2 = \gamma_3.$$

Then  $q$  depends on  $n, \gamma$  and  $u$ .

Show that the roots are given by

$$\lambda = -\gamma - q^{1/3}, \quad \lambda = -\gamma + q^{1/3}[\cos(\pi/3) \pm i \sin(\pi/3)] = -\gamma + q^{1/3}[1/2 \pm i\sqrt{3}/2] \quad (5.4)$$

Therefore  $\Re(\lambda) < 0$  for all 3 roots if and only if

$$q^{1/3}/\gamma < 2 \quad (5.5)$$

and  $\bar{x}$  is asymptotically stable in this case. If

$$q^{1/3}/\gamma = 2$$

there is one negative roots and two imaginary roots  $\lambda = \pm i\Delta$  and if

$$q^{1/3}/\gamma > 2$$

there is one negative root and a complex conjugate pair of roots with positive real part. In this case,  $\bar{x}$  is unstable. But remember that  $q$  depends on  $n, \gamma, u$ .

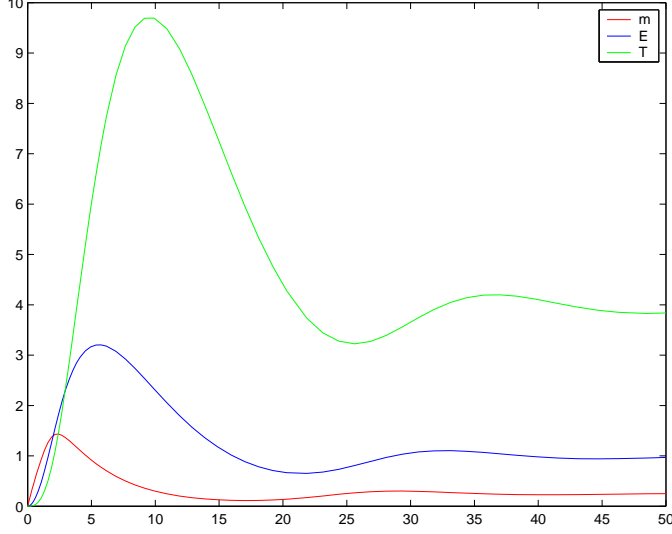


Figure 2: The time course of (4.2) with  $u = 0$ ,  $n = 2$ ,  $\gamma_i = 0.25$  and  $x_i = 0$  at  $t = 0$ .

Consider the case that  $u = 0$ . then use (5.1) and (5.3) to show that

$$q = n\gamma^6 x_3^{n+1} = n\gamma^3(1 - \gamma^3 x_3)$$

and  $\gamma^3 x_3 < 1$  by (5.1) so

$$q^{1/3}/\gamma = n^{1/3}(1 - \gamma^3 x_3)^{1/3} < n^{1/3}$$

implying that (5.5) holds if  $n < 8$ .

**Proposition 1.** *If  $u = 0$ ,  $n < 8$  and  $\gamma_i = \gamma > 0$ ,  $i = 1, 2, 3$ , then  $\bar{x}$  is asymptotically stable.*

Let's look for conditions making  $\bar{x}$  unstable for the case  $u = 0$ . Clearly, we must require that  $\frac{q}{\gamma^3} > 8$ . For simplicity of notation, let  $x = \bar{x}_3$ . Now,  $\gamma^3 = \frac{1}{x(1+x^n)}$  so the requirement becomes

$$\frac{1}{x(1+x^n)} < \frac{nx^{n-1}}{8(1+x^n)^2}$$

equivalently

$$\frac{x^n}{1+x^n} > \frac{8}{n}$$

Consequently, we see that  $n > 8$  is a necessary condition for instability and that we require

$$x > \left(\frac{8}{n-8}\right)^{1/n}$$

Hence we must have  $n > 8$  and, since  $\gamma^3 = \frac{1}{x(1+x^n)}$ ,

$$\gamma^3 < \left(\frac{n-8}{8}\right)^{1/n} \frac{n-8}{n} \quad (5.6)$$

**Proposition 2.** *If  $n > 8$  and (5.6) holds, then  $\bar{x}$  is unstable with two complex conjugate eigenvalues with positive real part and one negative eigenvalue.*

*Proof.* We must show that  $n > 8$  and (5.6) imply  $\frac{q}{\gamma^3} > 8$ . If  $u = \left(\frac{8}{n-8}\right)^{1/n}$  then (5.6) implies  $\frac{1}{x(1+x^n)} = \gamma^3 < \frac{1}{u(1+u^n)}$  so  $x > u$ . But this implies that  $8 < n \frac{x^n}{1+x^n} = n\gamma^3 x^{n+1}$  and on multiplying both sides by  $\gamma^3$  and noting that  $q = n\gamma^6 x^{n+1}$ , we find that  $8\gamma^3 < q$  as desired.  $\square$

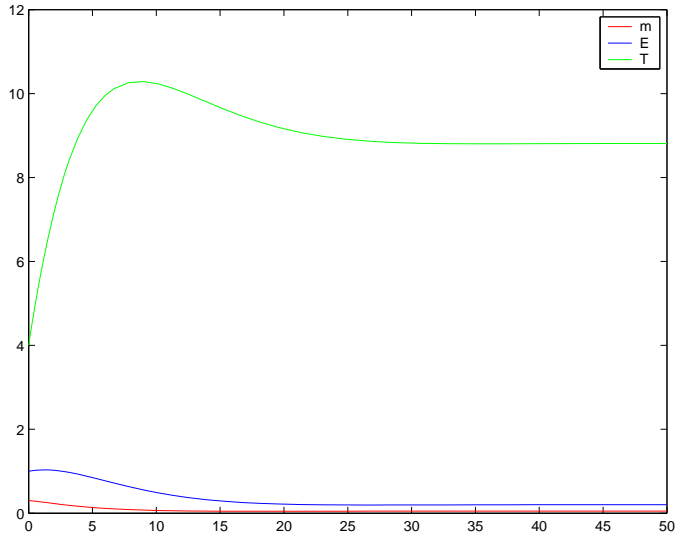


Figure 3: The time course of (4.2) with  $u = 2$ ,  $n = 2$ ,  $\gamma_i = 0.25$  and  $x_i$  set to their steady state values when  $u = 0$  at  $t = 0$ .

In particular, if  $n > 8$  then  $\bar{x}$  is unstable for all sufficiently small  $\gamma > 0$ .

Using Proposition 6.1 of [14], one can prove that most solutions are asymptotically periodic, that is, the omega limit set is a periodic orbit. See Figure 4 for a simulation with  $n = 10$  and  $\gamma = 0.1$ .

**Theorem 1.** *Let the hypotheses of Proposition 2 hold. Then every solution that starts off the one-dimensional stable manifold of  $\bar{x}$  is asymptotic to a periodic orbit.*

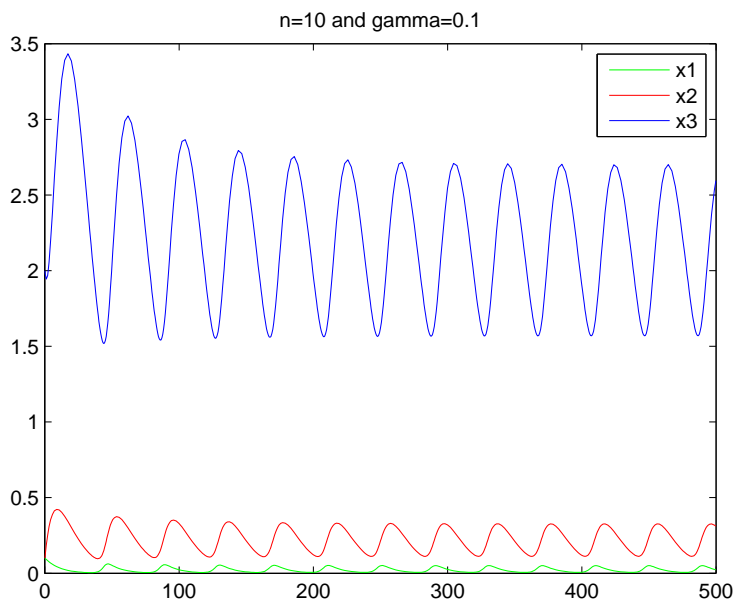


Figure 4: The time course of (4.2) with  $u = 0$ ,  $n = 10$ ,  $\gamma_i = 0.1$ .

## 6 Discussion

There is an enormous literature on the Goodwin oscillator and related equations. The references below contain references to much of this literature.

The Goodwin model can be applied to many other gene networks with positive and negative feedback. These systems often have more than one precursor molecule and the system has higher dimension. Here is a more general form.

$$\begin{aligned}x'_1 &= f(x_p) - \gamma_1 x_1 \\x'_i &= x_{i-1} - \gamma_i x_i, \quad 2 \leq i \leq p\end{aligned}$$

This system has positive feedback if  $f' > 0$  and negative feedback when  $f' < 0$ .

In his famous book [7], Murray uses the Goodwin model to model testosterone production in mammals.

The secant method [16, 15] was developed to determine the stability characteristics of matrices of the form

$$\begin{bmatrix} -\alpha_1 & 0 & \cdots & 0 & -\beta_1 \\ \beta_2 & -\alpha_2 & \cdots & 0 & 0 \\ \vdots & \vdots & \ddots & \vdots & \vdots \\ 0 & 0 & \cdots & \beta_p & -\alpha_p \end{bmatrix} \quad (6.1)$$

where  $\alpha_i, \beta_i > 0$ , that arise in determining the stability of steady states of the preceding equation. A sufficient condition that all eigenvalues have negative real parts is given by:

$$\frac{\beta_1 \beta_2 \cdots \beta_p}{\alpha_1 \alpha_2 \cdots \alpha_p} < \left[ \sec\left(\frac{\pi}{p}\right) \right]^p.$$

The article [8] shows that despite these systems having dimension higher than two, the Poincaré-Bendixson Theorem holds for them. As a consequence, it is shown that when the steady state has a pair of complex conjugate eigenvalues with positive real part, then there exists at least one stable periodic solution.

We have assumed that all the reactions described above occur instantaneously yet DNA transcription takes time, protein assembly in the ribosome also takes time. Therefore, it makes sense to include time delays in the equations leading to the system

$$\begin{aligned}x'_1(t) &= \frac{1}{1 + x_3(t - \tau_1)^n} - \gamma_1 x_1(t) \\x'_2(t) &= x_1(t - \tau_2) - \gamma_2 x_2(t) \\x'_3(t) &= x_2(t - \tau_3) - \gamma_3 x_3(t) + u(t)\end{aligned}$$

Time delays do not affect the steady state but do affect the stability of that steady state. See the references for more on models with time delays. In general, the external supply of tryptophan will be time dependent so we have included this by taking  $u$  time-dependent.

See Santillan and Mackey [11] for a more realistic model of the trp operon.

See the web site <http://www.che.eng.ohio-state.edu/~FEINBERG/RESEARCH/> for basic lectures on the differential equations of chemical reactions.

## 7 Homework Problems

# 1. Verify that the reference values in (4.1) may be chosen to obtain (4.2).

# 2. Verify (5.4).

# 3. Verify the computations leading to Proposition 1.

# 4. If we also assume that  $\frac{d}{dt}E = 0$ , then (4.2) simplifies to two equations. Analyze the phase plane and determine the asymptotic behavior of this system.

# 5. Show that  $\bar{x}$  is asymptotically stable for (4.2) if  $n = 1$ .

# 6. Show that there is a family of “Rectangles” of the form  $R(b) := \{x : 0 \leq x_i \leq b_i, 1 \leq i \leq 3\}$ , where  $b_i > 0$ , that are positively invariant for (4.2).

## 8 The Repressilator with 2 genes

The protein product of one gene can act to influence the rate of transcription of messenger RNA (mRNA) of another gene and thus act to control the expression of the other gene. Such a protein is referred to as a transcription factor. Its influence may be to down-regulate the expression of the other gene or to up-regulate its expression. Entire gene circuits, much like electrical circuits, have been discovered which control various facets of a cell physiology. See [10] for a well-written survey of some of these. This section is motivated by [6] where the authors, using genetic engineering, insert two genes on a plasmid into an *E. coli* cell, whose products act to down-regulate each other. The resulting circuit acts as a toggle switch, where there are potentially two stable steady states: one where one gene is “on” and the other “off” and vice-versa. They also construct a mathematical model of this circuit. The model below is a slight embellishment of their simple model, based on [9].

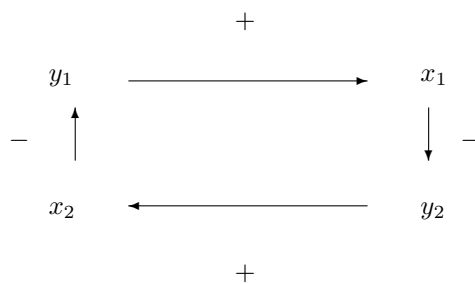
Consider two genes numbered one and two. Let  $x_i$  denote the protein product of gene  $i$  and  $y_i$  denote mRNA of gene  $i$ . We assume that  $x_1$  represses transcription of  $y_2$  and  $x_2$  represses transcription of  $y_1$ :

$$\begin{aligned} x_i' &= \beta_i(y_i - x_i) \\ y_i' &= \alpha_i f_i(x_{i-1}) - y_i, \quad i = 1, 2, \quad \text{mod } 2 \end{aligned} \tag{8.1}$$

where  $\alpha_i, \beta_i > 0$  and  $f_i > 0$  satisfies  $f_i' < 0$ . The  $f_i$  may have the Hill form:

$$f_i(x) = a + \frac{b}{1 + x^h}$$

This means that when  $x_1 \approx 0$ , then  $y_2$  is being transcribed at a high rate  $\alpha_2(a + b)$  but when  $x_1 \gg 1$  then the transcription is at the lower rate  $\alpha_2 a$ .



The Jacobian of (8.1) is given by

$$J = \begin{pmatrix} -\beta_1 & \beta_1 & 0 & 0 \\ 0 & -1 & \alpha_1 f_1' & 0 \\ 0 & 0 & -\beta_2 & \beta_2 \\ \alpha_2 f_2' & 0 & 0 & -1 \end{pmatrix} \tag{8.2}$$

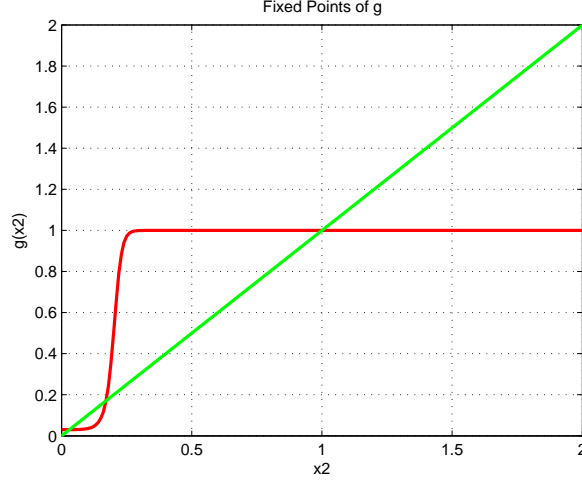


Figure 5: Graph of  $G$  (red) showing three fixed points

From the influence graph above,  $J$  is irreducible. Note also that it consists of diagonal  $2 \times 2$  quasipositive blocks and negative off diagonal  $2 \times 2$  blocks so (8.1) is already in canonical form for a cooperative irreducible system. The partial order generated by the cone

$$K = \{(x_1, y_1, x_2, y_2) \in \mathbb{R}^4 : x_1, y_1 \geq 0, x_2, y_2 \leq 0\}$$

is preserved by the solution map. Recall  $p = (x_1, y_1, x_2, y_2) \leq_K q = (\bar{x}_1, \bar{y}_1, \bar{x}_2, \bar{y}_2)$  if  $(x_1, y_1) \leq (\bar{x}_1, \bar{y}_1)$  componentwise and  $(x_2, y_2) \geq (\bar{x}_2, \bar{y}_2)$  componentwise.

It is easily seen that  $\mathbb{R}_+^4$  is positively invariant. The  $f_i$  are obviously bounded; let  $M = \sup\{f_i(x) : x \geq 0, i = 1, 2\}$ . Our usual differential inequality arguments give that

$$\limsup_{t \rightarrow \infty} y_i(t) \leq \alpha_i M, \quad \limsup_{t \rightarrow \infty} x_i(t) \leq \alpha_i M$$

Equilibria  $p = (x_1, y_1, x_2, y_2)$  must satisfy  $y_i = x_i$  and  $x_1 = \alpha_1 f_1(x_2)$ ,  $x_2 = \alpha_2 f_2(x_1)$ . Thus,  $x_1$  must satisfy

$$x_1 = (\alpha_1 f_1 \circ \alpha_2 f_2)(x_1)$$

We then get  $x_2 = \alpha_2 f_2(x_1)$  and the  $y_i$ . Denote  $G = (\alpha_1 f_1 \circ \alpha_2 f_2)$ . Clearly,  $G : [0, \infty) \rightarrow (0, \alpha_1 M]$  and it is strictly increasing.

Figure 5 depicts a case where  $G$  has 3 fixed points.

Hereafter, we make the following assumption about  $G$ :

$$G(x) = x \Rightarrow G'(x) \neq 1$$

This assumption implies that at an equilibrium  $p$

$$\det J(p) = \beta_1 \beta_2 [1 - G'(x_1)] \neq 0 \tag{8.3}$$

All equilibria are nondegenerate.

**Proposition 3.**  $G$  has an odd number of fixed points  $x_1^i, 1 \leq i \leq 2n + 1$ :

$$0 < x_1^1 < x_1^2 < \dots < x_1^{2n+1}$$

where  $G'(x_1^i) < 1$  for odd  $i$  and  $G'(x_1^i) > 1$  for even  $i$ .

If we denote by  $p^i, 1 \leq i \leq 2n + 1$  the equilibria, then

$$p^1 \ll_K p^2 \ll_K \dots \ll_K p^{2n+1}$$

because the  $x_1^i = y_1^i$  increase with  $i$  while the  $x_2^i = y_2^i$  decrease with  $i$ .

There is a beautiful and simple set of necessary and sufficient conditions for an  $n \times n$  matrix  $A$  with the property that  $P_m A P_m$  is quasipositive for some  $P_m = \text{diag}((-1)^{m_1}, (-1)^{m_2}, \dots, (-1)^{m_n})$ ,  $m \in \{0, 1\}^n$  to be stable. See [13]. Let  $B$  be the matrix obtained from  $A$  by replacing its off-diagonal entries by their absolute values. Then

$$s(A) < 0 \Leftrightarrow (-1)^k [k\text{-th principal minor of } B] > 0, \quad 1 \leq k \leq n$$

where the  $k$ -th principal minor is the determinant of the upper left  $k \times k$  block from  $B$ .

Using this test and (8.3) we get the following

**Proposition 4.** *The odd indexed  $p^i$  are asymptotically stable; the even indexed ones are unstable. If there is exactly one, it is asymptotically stable; in fact, it is globally asymptotically stable.*

*Proof.* A dissipative cooperative and irreducible system with a unique equilibrium is globally convergent to that equilibrium. To see this in our case, suppose  $z \in \mathbb{R}_+^4$  is a given point. We need only find points  $u$  and  $v$  in  $\mathbb{R}_+^4$  such that  $u \leq_K z \leq v$  and such that  $\omega(u) = \omega(v) = \{p^1\}$ . For this, just take a line segment in the positive direction with midpoint  $z$ : there are plenty of convergent points on this segment and the only equilibrium to which their orbit may converge is  $p^1$ .  $\square$

In order to simplify the analysis, we follow [9] by considering in detail the special case in which the two  $f_i$  have negative Schwarzian derivative. The Schwarzian derivative,  $SD(g)$ , of a scalar function  $g$  is defined as:

$$SD(g)(x) = \frac{g'''(x)}{g'(x)} - \frac{3}{2} \left( \frac{g''(x)}{g'(x)} \right)^2$$

The Schwarzian derivative has remarkable applications to the discrete-time dynamics of scalar maps. See especially [4]. The reader may easily check that if

$$f = a_i \frac{1}{1 + x^{h_i}} + b_i$$

where  $a_i, b_i > 0$  and  $h_i > 0$  then

$$SD(f)(x) = -\frac{h_i^2 - 1}{2x^2} < 0$$

if  $h_i > 1$

**Proposition 5.** *If the  $f_i$  have negative Schwarzian derivative,  $G$  has one or three fixed points.*

*Proof.* It's not hard to see (Prop 11.3, p.69, [4]) that  $SD(g) < 0$  and  $SD(h) < 0$  implies  $SD(g \circ h) < 0$  when the composition is defined. Consequently,  $SD(G) < 0$ . Observe that this means that any extrema of  $G'$  is a strict maximum ( $(G')'' < 0$ ). Therefore,  $G'$  can have at most one extrema and it must be a strict maximum because between any two maxima of a function, there must be a minimum.

Now  $G$  has an odd number of fixed points. If there exist at least 3 then since  $G'(x_1^1), G'(x_3^1) < 1 < G'(x_2^1)$  there must be an extrema of  $G'$  in  $(x_1^1, x_3^1)$ . If there were more than 3 then there are at least 5 fixed points and an argument as above implies that there would be an extrema in  $(x_1^3, x_5^1)$ , contradicting that there is at most one extrema. Therefore, we see that there are either one or three fixed points of  $G$   $\square$

We have already treated the case when (8.1) has a single equilibrium; below we consider the case of three equilibria.

**Theorem 2.** *Assume that (8.1) has exactly three equilibria  $p^1 \ll_K p^2 \ll_K p^3$  and let  $B^i = \{z \in \mathbb{R}_+^4 : \omega(z) = p^i\}$ . Then  $p^1$  is asymptotically stable and*

$$\{z \in \mathbb{R}_+^4 : z <_K p^2\} \subset B(p^1)$$

and  $p^3$  is asymptotically stable and

$$\{z \in \mathbb{R}_+^4 : p^2 <_K z\} \subset B(p^3)$$

The trajectory through almost every point of  $\mathbb{R}_+^4$  converges to one of  $p^1$  or  $p^3$ .

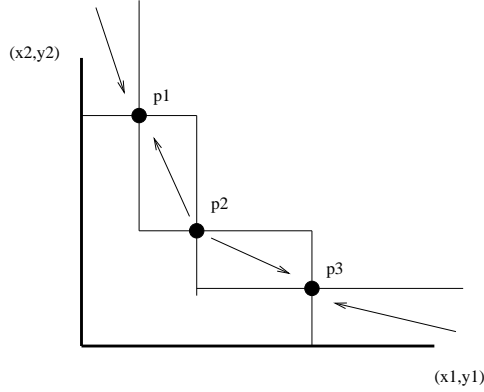


Figure 6: Phase portrait of (8.1) in case of three equilibria

*Proof.* Our general results show that  $B(p^1)$  contains all points  $z \leq_K p^1$  and all points  $z$  with  $p^1 \leq_K z <_K p^2$ . Any point  $z <_K p^2$  satisfies  $z_1 <_K z <_K z_2$  where  $z_1 <_K p^1$  and  $p^1 <_K z_2 <_K p^2$ . Since the trajectory through  $z_i$  converges to  $p^1$ , the trajectory through  $z$  does too. A symmetric argument gives the assertion concerning  $B(p^3)$ . The stable manifold of  $p^2$  is unordered (see Thm 2.10 [13]) and has measure zero.  $\square$

The toggle switch, as envisioned by Gardner et al [6], requires that there are three equilibria of which two are attractors. One attractor has gene one expressed at a high level (high  $x_1$ ) while gene two is essentially turned off (low  $x_2$ ); the other attractor being just the opposite. The switch is accomplished by an un-modeled effect: biologically, by transiently adding a molecule which inactivates the currently active repressor. For example, if the switch is in position  $p^1$ , where  $x_1$  is low and  $x_2$  is high, and we want to switch to  $p^3$ , one adds a molecule which binds to  $x_2$  making it unable to repress transcription of gene 1. It is most easy to see how this works if we assume

$$\beta_i \gg \alpha_i, 1.$$

In this case, we may assume that, after a short transient period,  $y_i \approx x_i$  so (8.1) reduces to

$$\begin{aligned} x_1' &= \alpha_1 f_1(x_2) - x_1 \\ x_2' &= \alpha_2 f_2(x_1) - x_2 \end{aligned} \quad (8.4)$$

Notice that the steady states and their stability properties remain the same as for (8.1). In addition, observe that (8.4) is a competitive planar system so all solutions converge to equilibrium.

Figure 8 shows the nullclines and equilibria for (8.4) where we have chosen

$$\alpha_i f_i(x) = 0.2 + \frac{5}{1 + x^2}, \quad i = 1, 2$$

The stable and unstable manifolds of  $p^2$  are included in green. Note the stable manifold is by symmetry, the diagonal  $x_2 = x_1$ .

Imagine that the system currently is in state  $p^1$  in the upper left where gene 1 is off and gene 2 is on. We want to switch to state  $p^3$ . We must introduce a molecule that binds to  $x_2$  and removes it. The effect should modify (8.4) by replacing the loss term  $-x_2$  in the second equation by,  $-cx_2$  where  $c > 1$ . Figure 7 shows the effects of this change where  $c = 2$ . Note now  $p^1$  and  $p^2$  are removed but  $p^3$  remains and solutions will be attracted to it. Now one shuts off the supply of the supplementary molecule so  $c = 1$  again and the system is now in state  $p^3$ .

Alternatively, nature may favor a single attractor at a time. For example, the molecule that binds to  $x_2$  and removes it may be produced for a long period of time so the state of the system is  $p^3$ . In order to switch to  $p^1$ , the cell first stops production of this molecule and subsequently begins production of an analogous molecule that binds to  $x_1$  and removes it.

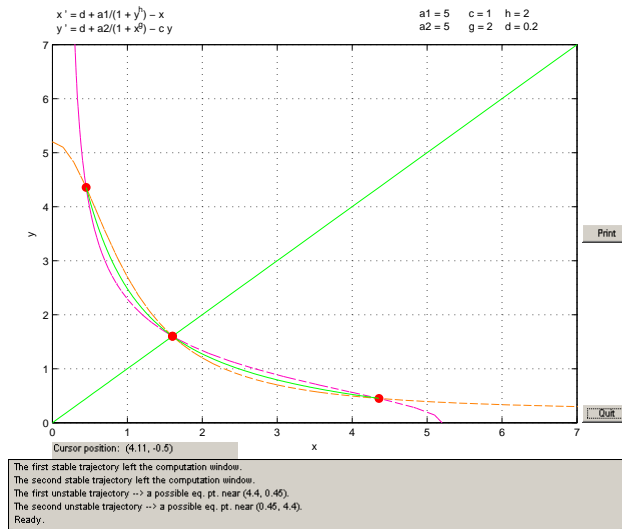


Figure 7: Nullclines of (8.4) and stable manifold (green) of saddle  $p^2$

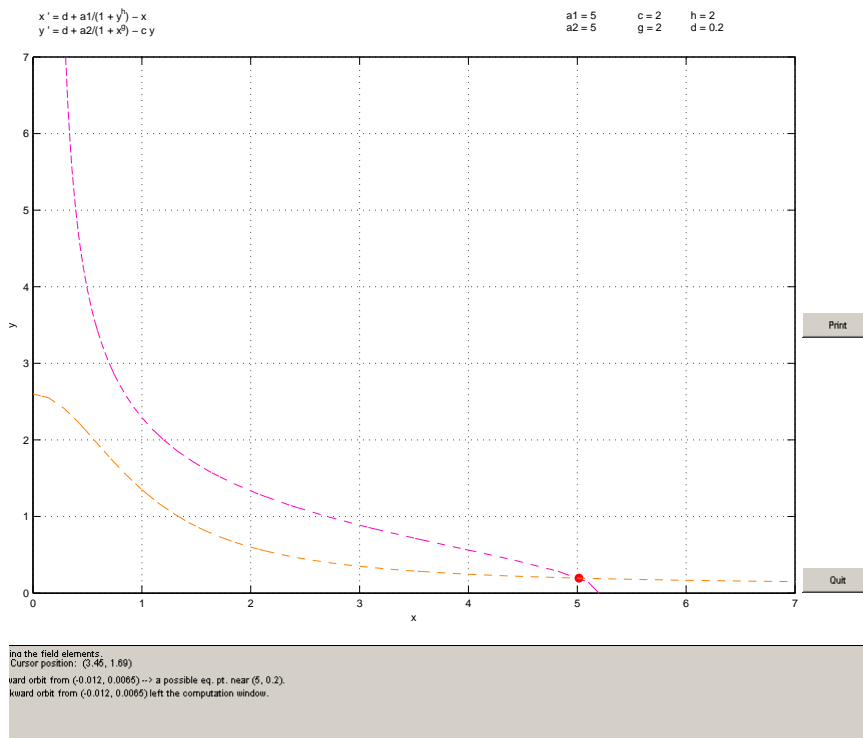


Figure 8: Modified Nullclines of (8.4)

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