Oct 21-23, 2014: Worksheet 14: Genetic Toggle Switch

(Throughout this tutorial, please fill in any blanks you find.)

Goal: To synthetically create a *bistable genetic toggle switch.* Explanation: by "genetic toggle switch," we mean a genetic system that can be used to switch on or off production of a specific protein. By "bistable," we mean the switch stays on once switched on, and stays __________ once switched off (even if the "inducer," or stimulus, that switched it __________ or off is removed).

In this tutorial, we look at the paper "Construction of a genetic toggle switch in *Escherichia coli*" (Nature vol. 403, January 2000), by Gardner et al. This paper describes a method of achieving the above goal. And check this out: the mathematics behind this method amounts, essentially, to a pair of differential equations and a set of initial conditions; that is, the mathematics amounts to an _____________ ____________ problem!!

1. **Setup.**

   Three different types of genes are implanted into a *bacteriophage,* which is a virus that infects a specific bacterium – in this case, the bacterium is *E. coli.* The three types are as follows:

   (a) A first "repressor gene" (so-called for reasons to be explained shortly). We will let u stand for the concentration of this first repressor gene, in μg/mL.

   (b) A second "repressor gene." We will let v stand for the concentration of this second repressor gene, also in μg/mL.

   (c) A "reporter gene," denoted GFP (again, the terminology will be explained shortly).

   Here's the **BIG IDEA**: The two repressor genes form a feedback mechanism – in particular, each repressor genes inhibits (slows down) transcription of the other. When the feedback results in a situation where $v > u$, the GFP gene is expressed, as a *green fluorescent protein.* (Hence the name "____________.") When the feedback yields $v \leq u$, there is no green fluorescent protein. (The glow is the "report" that tells us whether the switch is "on" or "__________.")

   The feedback mechanism described above can be encapsulated by the following two _________________________ equations:

$$(\mathbf{GT}) \qquad \frac{du}{dt} = \frac{\alpha_1}{1 + v^\beta} - u, \qquad \frac{dv}{dt} = \frac{\alpha_2}{1 + ku^\gamma} - v,$$

   where $\alpha_1, \alpha_2, \beta, \gamma,$ and k are positive constants (________________s). Let's analyze each the four terms in equations **(GT)**:

(i) The first term, $\alpha_1/(1+v^\beta)$, represents inhibition of the second repressor gene on transcription of the first. Indeed, as v gets larger, $1+v^\beta$ gets ____________, which means $\alpha_1/(1+v^\beta)$ gets ____________, which means (again, by the first of the **(GT)** equations) that du/dt gets ____________, which means u grows more ____________.

(ii) The second term, $-u$, represents degradation/dilution of the first repressor gene. In particular, this term tells us that degradation/dilution occurs at a rate that is ________________ to the concentration of this gene.

(iii) The third term, $\alpha_2/(1+ku^\gamma)$, represents inhibition of the first repressor gene on transcription of the ____________. Indeed, as u gets larger, $1+ku^\gamma$ gets ____________, which means $\alpha_2/(1+ku^\gamma)$ gets ____________, which means (again, by the second of the **(GT)** equations) that dv/dt gets ____________, which means v grows more ____________.

(iv) The third term, $-v$, represents degradation/dilution of the second repressor gene. In particular, this term tells us that degradation/dilution occurs at a rate that is ________________ to the concentration of this gene.

2. **Simulation.** Now we'll use MATLAB to model the behavior of the genetic toggle switch. Open up the Matlab m-file Toggle.m, which is set up to produce numerical solutions (using Euler's method) to the initial value problem consisting of

   - The differential equations **(GT)**; and
   - The ____________ conditions $u = v = 0.1$.

   When you call the m-file Toggle, you will need to designate specific values for the parameters (these include $\alpha_1, \alpha_2, \beta, \gamma, k$). If you read the comments below the function line in the m-file, you can cut and paste the needed commands into the Command Window that will pass the correct parameter values to the m-file. In what follows, we will be concerned only with changing the values for the parameter k.

   Note that the program is set up to graph u in green and v in blue.

   (a) The m-file initially is set with $k = 1$. Evaluate the code. What do you notice about the graph? With these starting conditions, will the green fluorescent protein be produced or not?

(b) Now we're going to simulate addition of a chemical called IPTG to the solution. Addition of IPTG has the effect of *decreasing* the parameter k. Let's suppose the new value of k is $k = 2.15672 \cdot 10^{-10}$. Change your call to the m-file to reflect this new value of k, and run the m-file again. What do you notice this time? Is the green fluorescent protein produced (eventually) or not?

(c) Based on what we've just seen above, we can think of IPTG as an *inducer* of green fluorescent protein: that is: addition of IPTG can turn our genetic toggle switch from __________ to __________.

(d) Because of what we said at the beginning of this worksheet about bistability, we should expect that, if we turn our switch __________ through addition of our ______________ IPTG, and then *remove* the _____________, our switch should remain __________ (at least, under appropriate conditions).

Let's check this, using our MATLAB m-file. *Here's how:* Immediately after the line that says

```
for n = 1:N
```

in your code, **remove** the percent signs that proceed the following three lines:

```
if dt*n>2
k=1;
end
```

This new code has the effect of telling MATLAB: For the first two hours, we'll go with $k = 2.15672 \cdot 10^{-10}$, meaning (as in exercise 2(b) above) that the solution contains _____________. But then, at $t =$_________ hours, we'll *remove* the _____________, meaning we now have $k =$_________, as in exercise 2(a) above.

(e) Run the new code you created in exercise 2(d) above, and comment on the stability of the switch (once it's in the "on" position).

(f) Replace your above line of code

```
if t>2
```

with

```
if t>1
```

Uh oh! What happened? What do you think this says about stability?