Math 1310: CLS **Name:** **SOLUTIONS**

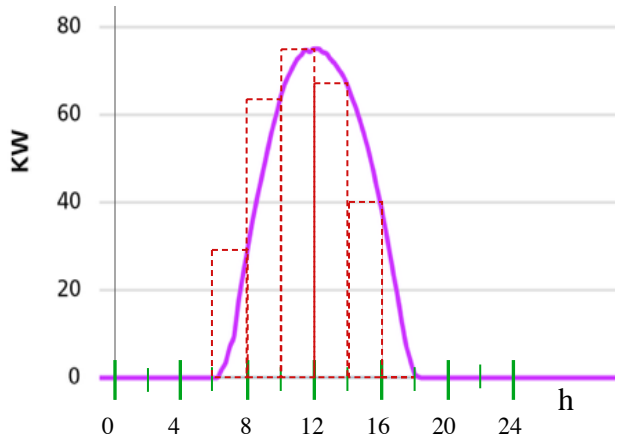
Individual Homework #7: Due in class Friday, March 15

Part A

Section 4.1 (pages 197–199):

2. (a) $1,000 \times 2 + 1,500 \times 3 + 500 \times 9 = 11,000$ watt-hours.

4. (a)



(b) $E(0) \approx E(2) \approx E(4) \approx E(6) \approx 0$, $E(8) \approx 60$, $E(10) \approx 60 + 128 = 188$, $E(12) \approx 188 + 152 = 340$, $E(14) \approx 340 + 136 = 476$, $E(16) \approx 476 + 80 = 556$, $E(18) \approx E(20) \approx E(22) \approx E(24) \approx 456$ (kilowatt-hours).

(d) To get a better approximation, use narrower rectangles for the approximation. For example, sample every half-hour instead of every two hours.

(e) $E'(T) = p(T)$, so the graph of E' looks like the original power function graph. (That is, it looks like the bell-shaped curve that you started with.)

Part B

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 that, under appropriate conditions, the switch stays on once switched on, and stays ___off___ once switched off (even if the "inducer," or stimulus, that switched it ___on___ or off is removed).

In this assignment, we consider the paper "Construction of a genetic toggle switch in *Escherichia coli*" (Nature vol. 403, January 2000), by Gardner et al. (There's a link to this paper in the "Some cool links" section of our web page. You don't *have* to read the paper to do this assignment, but it couldn't hurt, and you might find the article interesting.) 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 ___initial___ ___value___ 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 acronym "___GFP___.") When this happens, our switch is *on*. When the feedback yields $V \leq U$, there is no green fluorescent protein, and our switch is ___off___. (The glow is the "report" that tells us whether the switch is ___on___ or off.)

   The feedback mechanism described above can be encapsulated by the following two ___differential (or rate)___ equations:

$$\textbf{(GT)} \qquad \frac{dU}{dt} = \frac{\alpha_1}{1+V^{\beta_1}} - \gamma_1 U, \qquad \frac{dV}{dt} = \frac{\alpha_2}{1+kU^{\beta_2}} - \gamma_2 V,$$

   where $\alpha_1, \alpha_2, \beta_1, \beta_2, \gamma_1, \gamma_2$, and k are positive constants (in other words, they are ___parameter___s). Let's analyze each the four terms in equations **(GT)**. Fill in the blanks in parts (i)–(iv) below. Each blank should be filled in with one of the following words: larger; smaller; quickly; slowly; equal; proportional (some of these words may be used more than once, and some not at all).

   (i) The first term, $\alpha_1/(1+V^{\beta_1})$, represents the second repressor gene inhibiting (slowing down) transcription of the first. Indeed, as V gets larger, $1+V^{\beta_1}$ gets ___larger___, which means $\alpha_1/(1+V^{\beta_1})$ gets ___smaller___, which means (by the first of the **(GT)** equations) that dU/dt gets ___smaller___, which means U grows more ___slowly___.

(ii) The second term, $-\gamma_1 U$, represents degradation/dilution of the first repressor gene. In particular, this term tells us that degradation/dilution occurs at a rate that is ___proportional___ to the concentration of this gene.

(iii) The third term, $\alpha_2/(1+kU^{\beta_2})$, represents the first repressor gene inhibiting (slowing down) transcription of the second. Indeed, as U gets larger, $1+kU^{\beta_2}$ gets ___larger___, which means $\alpha_2/(1+kU^{\beta_2})$ gets ___smaller___, which means (by the second of the **(GT)** equations) that dV/dt gets ___smaller___, which means V grows more ___slowly___.

(iv) The fourth term, $-\gamma_2 V$, represents degradation/dilution of the second repressor gene. In particular, this term tells us that degradation/dilution occurs at a rate that is ___proportional___ to the concentration of this gene.

2. **Simulation.** Now we'll use Sage to model the behavior of the genetic toggle switch. Open up the Sage worksheet Toggle.sws (found under "The Sage Page" on our course page), 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 ___initial___ conditions $U = V = 0.1$.

The Sage code also designates particular values (taken from the article) for the parameters $\alpha_1, \alpha_2, \beta_1, \beta_2, \gamma_1, \gamma_2$, and k. In what follows, we will be concerned only with k.

Note that the program is set up to graph U in purple and V in green. *Remember that GFP is expressed when* $V > U$, meaning **GFP is expressed when the green curve is *above* the purple curve.**

(a) The worksheet 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? How can you tell? (You need not include a copy of this graph.)
No: as we see from the graph, $V \leq U$ for the entire duration.

(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 code to reflect this new value of k, and run the program again. (Careful: $2.15672 \cdot 10^{-10}$ should be typed in as `2.15672*10^(-10)`.) What do you notice this time? Is the green fluorescent protein produced (eventually) or not? (You need not include a copy of this graph.)
Yes: as we see from the graph, $V > U$ after enough time has elapsed.

(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 ___off___ to ___on___.

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

Let's check this, using our Sage code. *Here's how:* Immediately after the line that says

```
for i in range(length):
```

in your code, **add** the following lines:

```
    if t>2:
        k=1
```

Note: **the indentation is important here.** Here's how the added code should look with respect to what's already there:

```
for i in range(length):
    if t>2:
        k=1
    Uvalues.append(U)
```

This new code has the effect of telling Sage: 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 ___ITPG___. But then, at $t =$___2___ hours, we'll *remove* the IPTG, meaning we now have $k =$___1___, 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). *Please* **do** *include a copy of your graph here* (and also answer the above questions).

We waited until V was substantially larger than U, and then removed IPTG. We found that, although this caused a drop in V, it did not cause V to drop below U, even after significant time had elapsed. So yes, it does seem as though the switch is pretty stable (at least, in one direction).

(f) Replace your above new line of code

```
    if t>2:
```

with

```
    if t>1:
```

Uh oh! Did the switch stay on? Explain how you can tell.

What this says about stability is the following (fill in the blanks). Our switch is only stable under certain *conditions*. Specifically, what exercises 2(e) and (f) suggest is this: The switch does not necessarily stay on unless we *wait* for V to be quite a bit __bigger__ than U (not just a little bigger) before removing the __inducer__.

You're not quite done yet; there's one more question below.

3. **Wrap up.** Summarize in three to five (complete) sentences. What have we seen? What have you learned?

   A genetic toggle switch, meaning a genetic mechanism for turning on or off production of a specific protein, can be created through the interaction of two repressor genes. Each of these repressor genes inhibits growth of the other. IF IPTG is added, then one of the genes, call it gene 1, is inhibited more than the other, gene 2, and in this situation, a protein GFP is expressed. Even if the IPTG is then removed, GFP will continue to be expressed (as long as, at the point where the IPTG was removed, the concentration of gene 2 was substantially more than that of gene 1).