title: "Week 3 Problem setslass"
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DUE: Next Friday, beginning of class.

NOTES:

- All BIOE.80 problem sets must be completed individually unless plainly noted otherwise.
- Please turn in your completed problem sets as an electronic copy via Canvas.
- Please make sure to not go over the word limits and when appropriate show your work (e.g., calculations).

(Q1) Patterns with Logic

Your goal is to engineer patterns in tissues using proteins delivered by the hardware platform shown in Figure-1 (below). The platform itself consists of two channels (purple and green) through which proteins (ligands) are supplied continuously. A chamber in which engineered tissues develop (pink) sits between the two channels. Proteins from each channel can slowly move (diffuse) throughout the developing tissue. When the protein ligands are sensed by cells
within the tissue the cells respond according to whatever genetic logic is operating in each cell.

The concentration gradient of each protein ligand A and B is given in Figure-1. Note that values of A or B above the dotted line can be considered ‘1’ or ‘ON’, and values below can be considered ‘0’ or ‘OFF’.

Figure-1

Consider two different genetic logic devices (AND, XOR) that are responsive to proteins A and

B as inputs (“truth tables” or input/output relationship are given for each gate). Sketch the expected output (C) for each device as a function of the A and B inputs (draw on the figure below if useful).

Hint-1 For each logic gate use the corresponding truth table.

Hint-2 Each logic gate takes in two inputs, ligands A and B, then computes some output C to realize a spatially defined pattern across the population of cells (pink).

Figure-2 Cells with genetic logic devices-1: AND Gate.

Figure-3 Cells with genetic logic devices-1: XOR Gate.

(Q2) GOOP Tube (30 pts)

On Friday you were given two GOOP tubes, one tube of DNA (instruction to express GFP), and one tube of water.

Q.2.a. When and where did you add the water and DNA-expression to your GOOP tube.

Q.2.b. Did the GOOP with expression instructions turn green (produce GFP)? How long did it take? (Your grade does not depend on the expression of GFP). Include photos of both tubes at the start, about 6 hours and after 12 hours. (Time points don't have to be exact. Simply report when you took the photos) Make sure to provide a figure legend.

Q.2.c. Why might certain designs work in GOOP, but not in a cell? Why might certain designs work in a cell, but not in GOOP? (2-3 sentences)

Please give us feedback. Did you run into any problems?

(Q3) Bacterial edge detection (20 pts)

Building on work first published in 2005, Jeff Tabor and colleagues eventually demonstrated a bacterial edge detection system. In their system a bacterial lawn (i.e., a uniform layer of

identically engineered
bacteria growing on a plate) detect a light-encoded image. The bacteria are initially all the same but,
depending on whether they are exposed to light or not, send or receive small molecule-encoded signals that diffuse
across the light/dark boundary. Only cells positioned at the boundary between light and dark express an enzyme that
results in formation of a dark pigment (HINT: see Figure and also the primary source)

3.a. Develop a device-level block diagram that would result in the so-shown behavior. Label simple sensors, logic blocks, and actuators, as needed. Connect outputs to inputsif and as required. (Hint: Only give simple device names (e.g., “Dark sensor,” “pigmentactuator” etc.); do not describe any biology in any molecular detail). (30 points)

3.b. Take a look at the photo of the system in action. Note the edge detection in the case of asquare or Alfred Hitchcock’s portrait. What do you observe? For example, why is there more pigment inside the corners of the square, or inside the bottom left angle of Alfred’s portrait? (bullet points, 10 points)

(Q4) Approximate and rapid numerical estimates (40 pts)

Approximations based on simple physical principles are known as Fermi problems. These problems will help you build quantitative intuition for working with biology. Most of these estimates are rough: they are designed to give you a broad, order of magnitude intuition for the biology, so keep in mind that that the exactly correct number could be different. Give your answers to two significant figures and provide units where appropriate. You should only need simple math and arithmetic; a couple of lines of work at most.

Additional resources: bionumbers is an excellent source for biological numbers link . "*Cell Biology by the Numbers*" is a great book on estimation problems in biology. You can get a free copy of the draft version here.

Escherichia coli (Figure 1) is well-studied bacteria considered to be representative of how bacterial systems work generally (i.e., a 'model' organism). *E. coli* is also easy to grow in the lab and divides rapidly (about 20 mins). As a result, *E. coli* is frequently used in bioengineering either as a model organism (to be studied or engineered directly) or as a host to generate large copies of user defined DNA. You will see (and setup your own in BIOE44) cultures tubes of *E. coli* (with medium - food for bacteria) often growing overnight on shaking incubators (set to 37C) (Figure 2).

Figure 1. *E. coli* is shaped like a rod, which we can approximate as a spherocylinder: a cylinder with hemispherical caps. Of note, MG1655 is a strain derived from a lineage of *E. coli* variants that was originally isolated from a diphtheria patient at the Palo Alto hospital in 1922. Source TBD

Figure 2. *E. coli* grown to saturation (after 8-12 hours). *E. coli* divides about every 20 mins. Source: Cell Biology by the Numbers

Q.4.a. Based on a spherocylinder model, calculate the volume of an *E. coli* cell.

Q.4.b. If an *E. coli* cell were the size of a building, how big would a water molecule be?

Q.4.c. The mean diameter of a protein is roughly 4 nm. What is the upper bound on how many proteins could fit inside an *E. coli* cell?

Q.4.d. How would you go about estimating the total number of carbon atoms in an *E.coli* cell? You don't have to calculate this just define variables and describe your approach step by step.

Extra learning (100% optional):

If you want to explore more re: a different type of DNA logic mentioned briefly in class, pleasecheck out this video from Prof. Endy on how to to engineer Boolean integrase Logic (BIL) gatesbased on DNA flipping…
Transcriptors & Boolean Integrase Logic (BIL) gates, explained

(Q5) Ring Oscillator (0 pts)

Here is a system level diagram of a 3-inverter Ring oscillator. Think back to the class on Wednesday, imagine that you are a system's level Bioengineer designing an oscillator…

5.a. What is an ideal inverter from a systems level perspective? (bullet points and a simple diagram to describe inputs and outputs for the inverters) (5 points)

5.b. Provide an example of a bad design choice, from system's level view, for these inverters? (Ring oscillator should still work) (5 points)

(Q6) Buggy Bacterial Flash Mob? (0 pts)

Students at MIT are claiming that they have designed a genetic program to realize

arepeating bacterial flash mob. See their work yet again via:

Polkadorks, iGEM-2006

In an epic East Coast versus West Coast technology “battle,” some of your colleagues are now claiming that the genetic program designed at MIT won’t actually produce the so-desired behavior. Looking only at their proposed “device-level system diagram”…

1.a. What do you think? Will their program work or not? (10 points)

YES or NO (circle one)

1.b. Why or why not? (bullet points) (10 points)