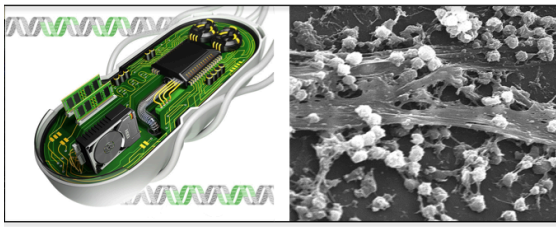
Lab 2. Synthetic Biology

AHL → LuxR → GFP



Introduction to Design Challenge: 3P (Penn Probiotic Pharma) has hired your team of bioengineers and synthetic biologists to create a new cell therapy platform, specifically a probiotic cell that can sense biochemical levels and respond by switching on the production of a biologic therapy (e.g. a cell that increases insulin at high sugar levels to treat diabetes). Ideally, these bacterial strains are tunable as a personalized medicine based on patient-specific dosing.

Your goal is to develop a mathematical model that predicts the response of the therapeutic genetic switch. As our model system, we will use engineered *E. coli* that sense acyl-homoserine-lactone (AHL) and express green fluorescent protein (GFP), an easily quantifiable reporter gene, in response (we call them "Receiver" cells). In these switches, AHL activates a transcriptional regulator, luxR, and activated luxR triggers transcription of the GFP gene. You will validate the model using various Receivers that have been tuned at the genetic level to have different transfer functions. By validating this model, you will have a powerful platform for designing new programmable cell therapeutics and for predicting their dynamic response to chemical inputs.

Your Design Challenge goal is for your model to meet the following requirements: (1) It is accurate, as measured by an average root mean-squared error (RMSE) value <0.125 when fit to experimental data on steady-state transfer functions, and < 0.2 Normalized RMSE (NRMSE) when fit to the 2D spatial patterning of gene expression induced by AHL. (2) Model parameters must be consistent with the underlying biology.

Overview of Sessions: We will do two structured wet lab exercises to acquire data to validate your model of your cells (dry lab). Both the data and models will be part of your Final Report.

1. (Day 1) MATLAB tutorial on finite difference approximations for solving differential equations.

2. (Days 2-3) Mathematical modeling and testing the steady-state transfer function of the engineered genetic switches.

3. (Days 4-5) Design Challenge – Create a predictive model of dynamic (non-steady state) spatial patterning of gene expression, and test its accuracy experimentally.

Track	Day 1	Day 2	Day 3	Day 4	Day 5
Wet Lab	None	Induce Receiver strains	Measure steady-state transfer functions.	Test dynamic patterning of gene expression	Analyze Day 4 data.
Dry Lab	MATLAB Models, problem set	Model transfer functions for Day 3	Finish model and fit to data	Model dynamic patterning of gene expression	Fit Day 4 data and refine. Module quiz.

Relevant Assignments
1. Individual problem set
2. Interim report
3. Final lab

Consult Canvas and the class schedule for due dates & times.

Day 2. Inducing Receiver Strains with AHL and Building Steady-State Models

INTRODUCTION

In this lab session, you will measure and mathematically model the transfer function of a genetic switch. In our case, you will model the relationship between ligand concentration (the input, AHL) and green fluorescent protein reporter gene expression (the output), which is induced by the AHL-responsive LuxR transcriptional activator. This model will be based on enzyme kinetics and is useful for mathematically describing a biological system in a "dry lab" to predict cellular behavior. To fit this model to empirical data, we will measure GFP expression in engineered bacterial strains (the "Receivers") in response to known concentrations of AHL. Today you will induce expression in these bacteria, and you will measure the resultant GFP expression in the next laboratory session.

OBJECTIVES

1. To introduce critical concepts in synthetic biology and cellular engineering, namely the transfer function of chemical-sensing genetic switches or transcriptional circuits.
2. To build mathematical models of ligand-induced gene expression, which is useful for linking experimental data with theory, and as a "dry lab" to rapidly explore/predict how different parameters govern the behavior of cells.
3. To induce Receiver strains with AHL in preparation for measuring their gene expression transfer functions in the next laboratory session.

BACKGROUND

- Review your BIO121 and BE324 notes on the central dogma, gene regulation, operons, and the Hill Equation.
- Alon, Uri. "Chapter 2 – Transcription Networks: Basic Concepts" in An Introduction to Systems Biology: Design Principles of Biological Circuits
- Canton et al. "Refinement and standardization of synthetic biological parts and devices." *Nature Biotech.* (2008), 26, 787-93 [*background on key synthetic biology principles*]

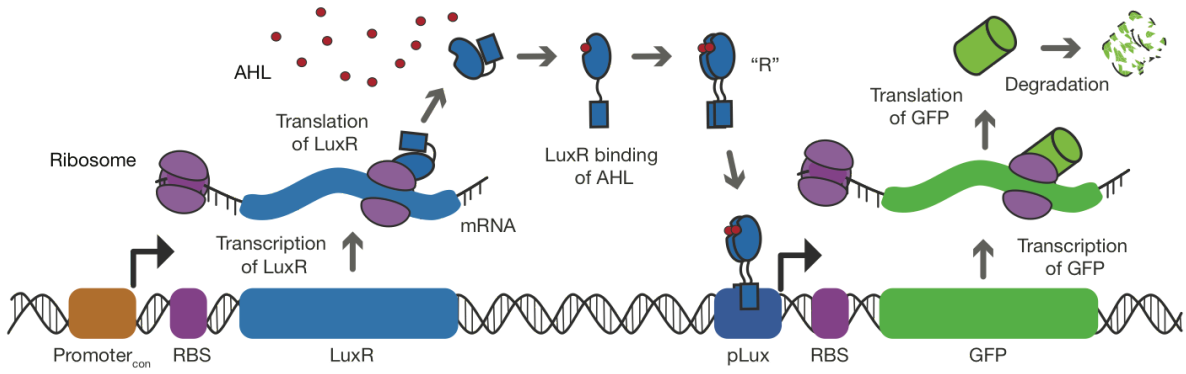
APPARATUS, MATERIALS, AND EQUIPMENT (Lab day 2)

- NEB Turbo Cells (*E. coli*) pre-grown to OD ~ 0.6 in M9 media + Chloramphenicol
    - [50 mL each] S1, S2, and S3 Receiver Cell Cultures
    - [300 µL] Stock of 10 mM N-(3-Oxohexanoyl)-L-homoserine lactone (AHL)
- [10 mL] Sterile-filtered H2O
- [2] 48-well deep well plates and [2] Breathe-easier sealing strips
- Gloves, Micro-Pipetters, Serological Pipetter, autoclaved microcentrifuge tubes, beakers, and Lab notebook

METHODS The session includes wet lab and MATLAB coding. ***Chemical inductions must be completed by the end of the session. Modeling can be completed after class if necessary.***

Wet Laboratory - AHL Induction of LuxR in Receiver Strains: Here, we will induce GFP reporter gene expression in engineered bacterial Receiver strains. In our genetic circuit (**Figure 1a**), the GFP reporter protein is under the transcriptional regulation of LuxR protein, which is activated by the ligand acyl-homoserine lactone (AHL). Thus, AHL induces GFP expression. The strains are engineered at the genetic level to exhibit different transfer functions (**Figure 1b**). Specifically, strains will differ in two parameters: "Promoter$_{con}$" strength and "R" strength. Promoter$_{con}$ is the DNA region that regulates transcription *of* luxR (not to be confused with pLux, the DNA binding target of activated luxR). The strain with "high" Promoter$_{con}$ has a promoter region sequence with a higher affinity for *E. coli* RNA polymerase. "R" strength defines the strength of binding of the luxR protein to its target promoter, pLux. The strain with "high" R expresses a mutant R that binds to pLux with greater affinity than the others. We will further explain the engineering in lecture.

Promoter$_{con}$ → LuxR ↑ → GFP↑ "R" → GFP ↑



Relative strengths of engineered strains

Strain #	***Promoter-con strength***	***R strength***
S3	Low	High
S2	Low	Low
S1	High	Low

Figure 1. Overview of strain design. (Top) Schematic representation of the molecular events that give rise to the transfer function of a genetic switch or transcriptional circuit. LuxR protein expression is under the control of a constitutive promoter ($Promoter_{con}$) and a ribosome binding site (RBS). Upon binding the AHL ligand, LuxR protein dimerizes to form the active "R," which binds the pLux promoter to activate transcription of GFP-encoding mRNA. GFP reporter protein is synthesized after ribosomal translation of the transcript and eventually degrades. AHL is the input and GFP expression is the output of the transfer function. **(Bottom)** Differences between the engineered strains, S1-S3, which vary in $Promoter_{con}$ affinity of E. coli RNA polymerase (engineered by mutating the $Promoter_{con}$ DNA sequence) or the affinity of activated R transcriptional activator to its target promoter (engineered by mutating the DNA sequence encoding for the promoter-binding pocket of LuxR protein). For any parameter, "Low" in one strain would have the same DNA sequence as "Low" in another (and similarly for "High").

M1. Get two 250 mL or larger beakers, labeling one "Used Tips", and the second, "Liquid Waste"

M2. Serially dilute the 10 mM AHL stock from 10 mM to 100pM, at every log order (~200 µL for each working concentration, 10 total solutions). Use sterile water (not DI water). Use the provided autoclaved microcentrifuge tubes, and make sure they are clearly labeled.

M3. Plan how you will prepare your samples in 48-well plates. **Appendix 1** (separate file on Canvas) has printable diagrams to help track samples. You have 3 different receiver strains, each to be induced at 10 AHL concentrations (including 0 µM negative control) in **duplicate**. You will induce 2 mL of each strain with 20 µL of AHL working solutions. How many samples do you need and what are the final [AHL] concentrations in the bacterial cultures?

M4. Label plates as GXX-N, where XX is your group number and N is the plate number.

M5. Dispense 2 mL of bacteria into each well following the M3 layout. You can reuse a pipette to dispense the same strain into different wells, but ***must change pipette tips between strains to avoid contamination.***

M6. Induce each Receiver with 20 µl of each AHL solution according to your M2 plan. Change pipette tips with every sample to avoid contamination.

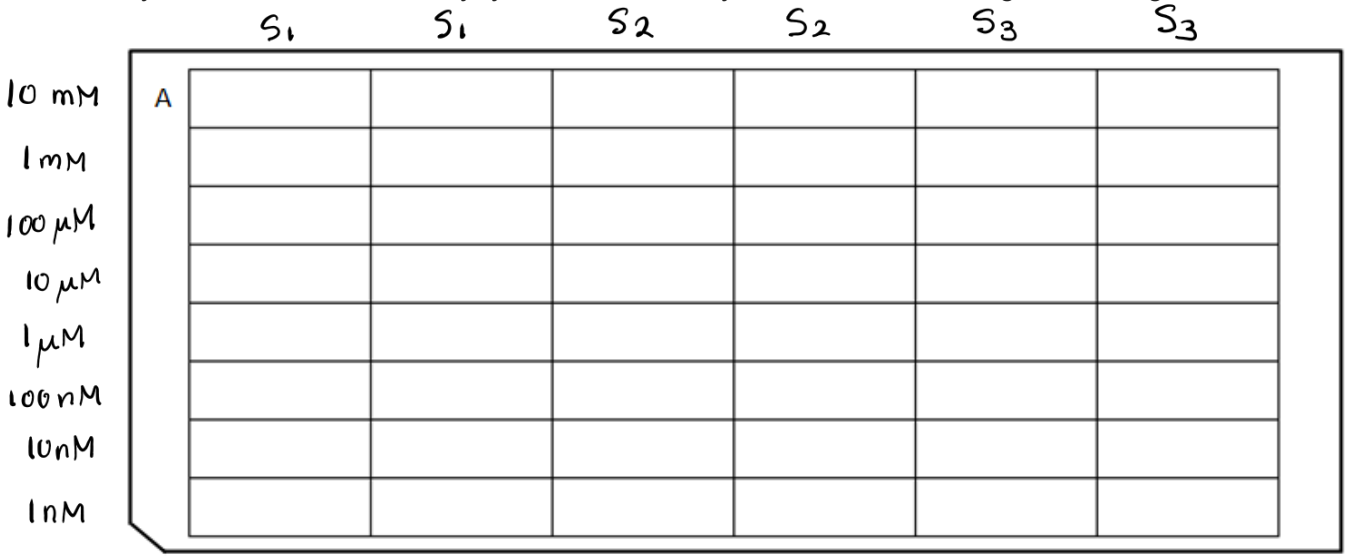
S_1, S_2, S_3 in duplicates.

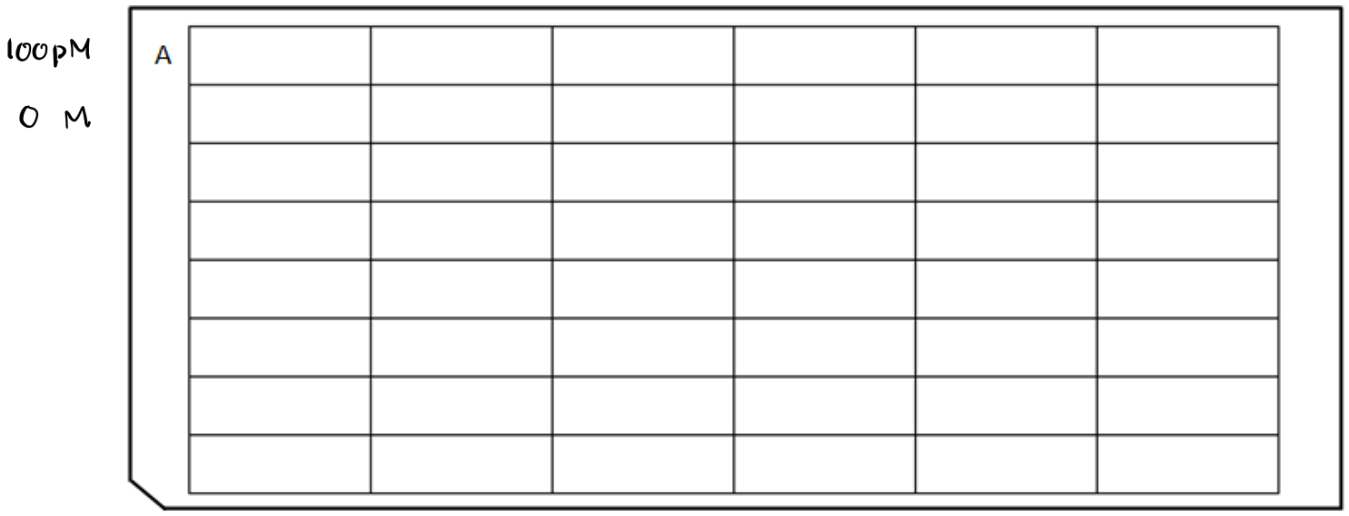
20 µl each 2mL of strain ×20

120 µl of each conc. 40 mL of strain

Appendix 1: Liquid culture sample tracking templates

To prevent sample mix-ups, you should write on these and cut-and-tape them into your laboratory notebooks. Alternatively, you should make your own tables for digital tracking.





Label	[AHL] Stock	Final [AHL] in Well
	0 M	
	100 pM	
	1 nM	
	10 nM	
	100 nM	
	1 µM	
	10 µM	
	100 µM	
	1 mM	
	10 mM	

M7. Place the sealing strip over each plate, completely sealing each well. Leave the plates on your bench for lab staff. The plates will be shaken at room temperature (~21 ºC, 250 RPM) for 20 hours, and then placed in a 4 ºC fridge until the next lab session. We store the strains at 4 ºC to prevent overgrowth between lab days. It minimally impacts results because GFP expression reaches steady state during shaking. ***For the final report methods, you should write as if you conducted this step yourself***

M8. Transfer all excess liquid into the "Liquid Waste" container. Leave this at your station.

M9. Dispose of the contents of the "Used Tips" container into a Biohazard bin. Remove the label, wash, and put away the container. All test tubes and microcentrifuge tubes (empty of liquids) can be disposed into a Biohazard bin.

Dry Laboratory – Modeling the transfer function of the Receiver. Here, you will model Receiver transfer functions, which we derived in lecture.

M1. Build a model from three ODEs below. Because expression will reach steady state by the time of measurement (next lab), solve the ODE's at steady state with an output in concentration (intracellular protein concentrations are typically 1 nM – 1 μM, but can reach the mM range when overexpressed). Use the function 'semilogx' for plotting your model in log[AHL]. How do the parameters alter switch point, baseline, range, and steepness of the sigmoid?

a. **Activation** of LuxR by AHL to create the pLux-binding activated dimer 'R' (Eq. 1).

$$EQ\ 1: \frac{d[R]}{dt} = \left(\rho_R[LuxR]^2[AHL]^2 - \delta_R[R]\right)$$

b. Concentration of **mRNA or transcript** encoding for GFP (TX_{GFP}), based on the **transcriptional activation** by R, and the degradation of TX_{GFP} (after its synthesis):

$$EQ\ 2: \frac{d[TX_{GFP}]}{dt} = \left(\frac{\alpha_{TX_{GFP}}\left(\frac{[R]}{K_R}\right)^{n_1}}{1+\left(\frac{[R]}{K_R}\right)^{n_1}}\right) - \delta_{TX_{GFP}}[TX_{GFP}]$$

c. GFP **protein** concentration based on the **translation** of TX_{GFP} and the degradation rate of GFP protein (after its synthesis).

$$EQ\ 3: \frac{d[GFP]}{dt} = (\alpha_{GFP}[TX_{GFP}] - \delta_{GFP}[GFP])$$

Suggested Starting Point for Constants(*):

LuxR protein concentration	[LuxR] = 0.1μM	Synthesis rate constant of TX_{GFP} after R-binding to pLux	α_{TXGFP} = 0.05 μM/min
Formation rate constant of dimeric R protein from LuxR protein binding to AHL	ρ_R = 0.5μM^{-3} min^{-1}	Degradation rate constant of TX_{GFP}	δ_{TXGFP} = 0.2 min^{-1}

Degradation rate constant of dimeric R protein	δ_R = 0.0231 min^{-1}	Synthesis rate constant of GFP protein	α_{GFP} = 2 min^{-1}
Transcriptional activation threshold of dimeric R binding to the pLux promoter	K_R = 1.3e-5 μM	Degradation rate constant of GFP protein	δ_{GFP} = 4e-4 min^{-1}
Hill coefficient (**)	n_1 = 1		

(*) Previously provided AHL diffusion and degradation constants can be changed.
(**) The Hill coefficient should not change. It has been experimentally determined by others to be 1.

ANALYSIS AND DISCUSSION
These topics should be covered in your final report.

A1. Based on the differences in genetic circuit design between strains, which variables would be biologically valid to change independently of one another, and which variables must be changed in concert (but can be different from the suggested starting point parameters)?

A2. Why was your starting sample concentration OD ~ 0.6, or mid-log phase of cell growth?
Best growth → Happy

A3. Why are your strains grown in the presence of chloramphenicol, which is an antibiotic?
Plasmid w/ resistance

Day 3. Measuring the Steady-State Transfer Function in Liquid Culture

INTRODUCTION You will measure the AHL-to-GFP transfer functions of the induced Receivers using optical spectroscopy, and you will fit the data to the mathematical model to refine the constants. The mathematical models will later be combined with models of ligand diffusion in 2D to build a model of dynamic 2D spatial patterning of gene expression during the Design Challenge.

OBJECTIVES

1. To measure the transfer functions of genetically engineered strains of bacteria using a multi-mode spectrophotometer.
2. To establish a model system for chemical sensing or dynamically responsive bacterial drug delivery systems.
3. To bridge biology and math through data-informed mathematical modeling that describes and predicts the behavior of complex biological systems.

BACKGROUND See Part 1 and SpectroVisPlus instructions (on BE Lab website)

MATERIALS AND EQUIPMENT (Lab day 3)

- [2] Vernier SpectroVisPlus Spectrophotometer (instructions on BE Lab website)
- 1.5 mL cuvettes
- Induced Receiver Cultures from previous session
- [2] 50 mL aliquots of M9 media to be used for dilution of samples
- Gloves, Micro-Pipetters, Serological Pipetter, beakers, and Lab notebook

METHODS As you complete today's work, please take note of the deliverables requested in the Analysis and Interim Report.

M1. Get two 250 mL or larger beakers, labeling one "Used Tips", and the second, "Liquid Waste"

M2. Mix the returned samples with a pipette (pipet up and down) to resuspend bacteria that have settled to the bottom before taking a measurement. Make sure to use a new pipette tip for each sample to avoid contamination.

M3. Measure GFP fluorescence ($\lambda_{Excitation}$ = 500 nm, $\lambda_{Emission}$ = 540 nm) in fluorescence mode, and optical density in "absorbance" mode (λ = 600 nm), for each Day 2 culture. Use the guide available on the lab website for using the spectrophotometer. Consider the following:

   a. Excessive exposure to bright light (ambient or while the spectrophotometer is on) photobleaches GFP. How does this impact the accuracy? How can you avoid it?
   b. It is not easy to switch between device modes, so measure all ODs for one strain, and then all fluorescence (or, use one device for ODs and the other for fluorescence).
   c. For Absorbance measurements, set the sampling duration to 4 seconds, and the sampling rate to 2 samples/second. What will you use as your blank? Why? Check with lab staff, instructors, or TAs before proceeding – be prepared to answer "why?".
   d. For Fluorescence measurements, you must first set the scale for the curves, which is determined by the highest fluorescing sample per strain. Setting the measurement integration of this sample to nearly saturate the device detector maximizes the dynamic range of your assay (similar to setting exposure time of a camera).

i. Set up the spectrophotometer for Fluorescence (Steps 1 & 2 for Fluorescence of the Guide on the website).
ii. Open the setup menu: Experiment Set Up Sensors Spectrometer: 1. The setting, “Sample Time [15 to 1000]” will be set to the default of 50 ms.
iii. Adjust the integration time:
    1. Identify the strain with the highest fluorescence (greenest wells for each strain)
    2. Take measurements, as described in the Guide on the website, and adjust the Sample Time so the fluorescence value is 0.75-0.9 (i.e. high but below the saturation)
iv. Now you are ready to collect fluorescence data.

M4. Generate a transfer function for each strain by plotting average fluorescence (in relative units) per O.D. for each concentration of AHL. Use the zero-concentration point for each strain to subtract background auto-fluorescence of the cells and media. The x-axis should be in log scale and the shape should be sigmoidal.

M5. Show your plot to a staff member before you leave class (including axes, units, and error bars), so we can make sure experiments do not need to be redone**.**

M6. Transfer all excess liquid into the “Liquid Waste” container. Leave this at your station.

M7. Dispose of the contents of the “Used Tips” container into a Biohazard bin. Remove the label, wash, and put away the container. All test tubes, cuvettes, and microcentrifuge tubes (empty of liquids), and the deep-well plates can be disposed into a Biohazard bin.

M8. Calculate RMSE in Excel or MATLAB loop. Adjust parameters to minimize it. To fit the model to data, all values need to be on the same scale. Therefore, normalize each transfer function to its maximum value (both for the model and data).

$$EQ\ 4: RMSE = \sqrt{\frac{1}{n}\sum_{i=1}^{n}\left(Y_i^* - Y_i\right)^2}$$

where Y_i^* and Y are respectively model and data values at a given AHL concentration and n is the total number of AHL concentrations

ANALYSIS AND DISCUSSION

A1. How do the transfer functions vary in their switch points (mid-point of sigmoid), baseline expression, expression range, and steepness? How do these features agree/disagree with your model or expectations? What are the possible explanations for disagreement?

A2. What features does the model predict well/poorly? How can it potentially be improved?

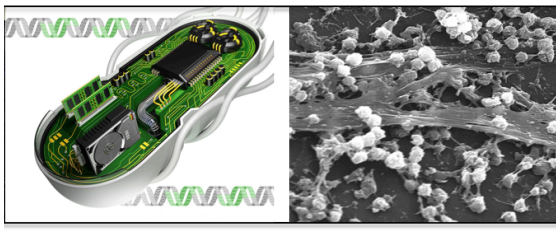
A3. What is a shortcoming of normalizing the data to max, when making comparisons between strains? How would the shortcoming(s) impact drug delivery function?

A4. You can remove data points of apparent outliers from the sigmoidal fit, but you should (i) still report all the data points in your graphs, and (ii) explain in your methods your quantitative reason for exclusion from the fit. Your exclusion criteria will be examined carefully.

Day 4-5. Design Challenge

OBJECTIVES

Integrate lessons learned in previous sessions and apply them to a design challenge.



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Your goal is to develop a mathematical model that predicts the response of the therapeutic genetic switch. As our model system, we will use engineered *E. coli* that sense acyl-homoserine-lactone (AHL) and express green fluorescent protein (GFP), an easily quantifiable reporter gene, in response (we call them "Receiver" cells). In these switches, AHL activates a transcriptional regulator, luxR, and activated luxR triggers transcription of the GFP gene. You will validate the model using various Receivers that have been tuned at the genetic level to have different transfer functions. By validating this model, you will have a powerful platform for designing new programmable cell therapeutics and for predicting their dynamic response to chemical inputs.

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IMPORTANT*: *You cannot use a MATLAB/Python solver function.

APPARATUS AND EQUIPMENT (Design Challenge)

- NEB Turbo Cells pre-grown in M9 media + Chloramphenicol
  - [4 mL] of one strain (S1, S2, or S3) grown to OD ~ 0.6
- [2] M9 Media Plates supplemented with Chloramphenicol
- [4 mL] of 0.7% Agarose solution (2 mL aliquots), located in 55ºC Hot Water Baths
- [2] Filter paper discs (6mm diameter) in an empty petri dish
- [10 mL] Sterile-filtered H_2O
- [100 µL] Stock of 10 mM N-(3-Oxohexanoyl)-L-homoserine lactone (AHL)
- [2] Sterile Forceps
- Liquid Waste Beaker, Kim Wipes, Pipettes, Tube Rack, Black tape

METHODS. You will test and model the spatial patterning of gene expression in 2D on bacterial plates uniformly coated with Receivers that will fluoresce when induced by AHL diffusing from a localized source (soaked filter paper). The model will be built from your existing models of gene expression and diffusion.

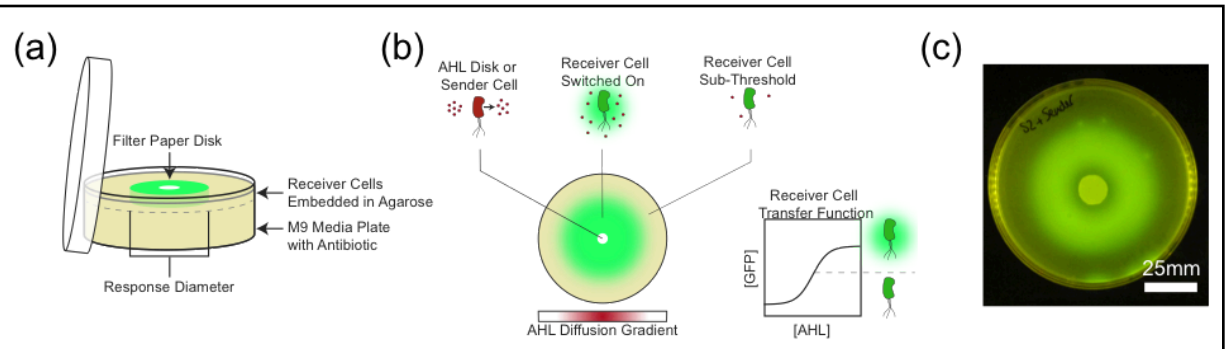


Figure 1. Overview of spatial patterning of gene expression experiment. **(a)** 3D geometry of the bacterial plate. A thin layer of Receivers uniformly coats the top of the plate, and a filter paper soaked in AHL sits on top. **(b)** Top view of the experiment. Spatial patterning of GFP expression is governed by AHL diffusion from the disk and the transfer function of the switch. **(c)** Representative image of the experiment after 24 hours.

M9 Plate Preparation and Receiver Induction

You will be performing the plating exercise outlined below for one of the Receiver Strains. This is an exercise in how the supplied images were generated.

M1. Get two 250 mL or larger beakers, labeling one “Used Tips”, and the second, “Liquid Waste”

M2. Get two M9 plates from the 37° C incubator. Label the bottoms with GXX-R# (XX = group #). Make the labels small and near the edge to not interfere with imaging.

M3. Create a thin agarose layer of Receivers on top of each M9 plate. ***M3 must be done quickly so the agarose does not solidify (c-d ~<30 seconds).*** Make one plate at a time.

a. Bring your plates over to the plating station near the water bath.
b. Take a 2 mL aliquot of 0.7% agarose solution from the water bath
c. Pipette 2 mL of Receiver into the agarose solution aliquots (one bacteria-agarose solution per Receiver). Pipet up and down a few times to mix.
d. Pour out the solution onto the corresponding plate. Tilt plate side-to-side and in a circular motion to create an even layer of agarose.
e. Allow the agarose layer to dry for ~15 minutes.
f. Repeat for the second plate

M4. Dilute the 10 mM AHL to 10 µM.

M5. Add 10 µL of 10 µM AHL to each disc and allow them to dry.

M6. Use sterile forceps to place filter discs in the center of each plate.

M7. Wipe off any condensation on the plate lid with a Kimwipe. Flip the plate so that the lid is now on the bottom to prevent condensation.

M8. Show the Teaching Staff your two finished plates.

M9. Transfer all excess liquid into the “Liquid Waste” container.

M10. Dispose of the contents of the “Used Tips” container into a Biohazard bin. Remove the label, wash, and put away the container. All test tubes, microcentrifuge tubes (empty of liquids), and replacement petri dishes and M9 plates, can be disposed into a Biohazard bin.

Image Acquisition and Analysis: Images are acquired on an automated fluorescence imager that consists of a Raspberry Pi computer and camera synced to a transilluminator (we will discuss its workings in lecture). The system allows you to continuously acquire ~24-hrs data between class sessions without being present. ***You will be given access to a folder on Google Drive that has the time lapse images of this experiment for all three strains. The plates in the photo are 85 mm in diameter. This is different from the plates used in lab.***

M1. Use the MATLAB image toolbox to measure the observed GFP edge distance from the center of the disk (you can assume the soaked disk is uniform in AHL concentration). You may determine center and edge by eye. The functions *imdistline* (distance between two points) or *improfile* (pixel values in a line section) may be helpful. Alternatively, you may use ImageJ if you prefer, based on your past experience.

- Plot GFP visualized edge distance (mm) from the center of the disk vs. time (hours) for each strain, at least once every 3 hrs.
- Use the diameter of the **disk** to convert pixels to millimeters.
- ***Different camera exposures can be used for different strains, but each strain must be analyzed with the same camera exposure.***

Model Development: To model the plate experiment, you will combine a 2D diffusion model with your transfer function. ***You may use a square boundary for the plate.***

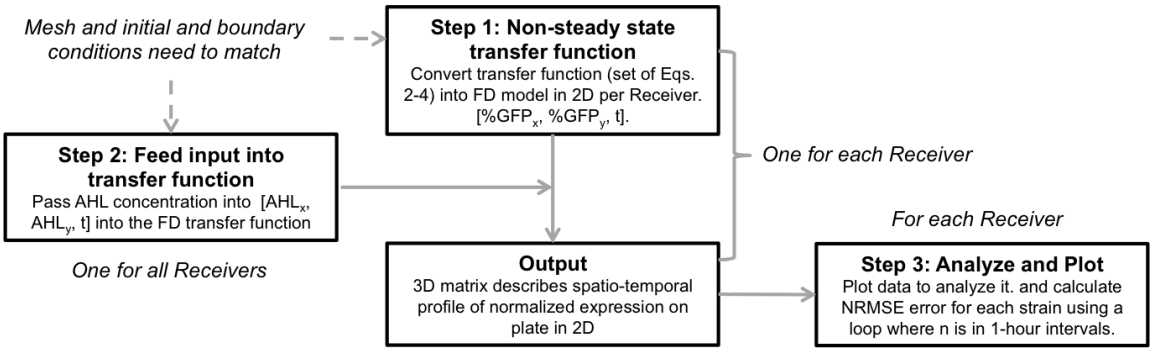
M1. Create a finite difference model for diffusion in 2D, described by Figure 1 and Eq. 4:

$$EQ\ 5: A_{x,y}^{t+1} = A_{x,y}^{t} + \frac{\Delta t}{\Delta p^2} D\left(A_{x-1,y}^{t} + A_{x+1,y}^{t} + A_{x,y-1}^{t} + A_{x,y+1}^{t} - 4A_{x,y}^{t}\right) - \Delta t(d_{AHL} * A_{x,y}^{t})$$

Plot modeled edge distance vs. time up to 21 hours. **Define the AHL edge as where AHL concentration is equal to your steady-state switching point.** Here, we assume

$\Delta x = \Delta y = \Delta p$ = step size.

M2. Create a finite difference model of the dynamic spatial patterning of gene expression, by making a non-steady state model of gene expression and passing in time-varying AHL concentration profiles from a 2D diffusion model.



M3. Plot modeled GFP edge distance vs. time up to 21 hours in 3-hour intervals.

M4. Adjust your parameters to best fit your model to your experimental data (edge distance vs. time) for each strain by minimizing NRMSE:

$$EQ\ 6: NRMSE = \frac{RMSE}{y_{max} - y_{min}} = \frac{\sqrt{\frac{1}{n}\sum_{i=1}^{n}\left(Y_i^* - Y_i\right)^2}}{y_{max} - y_{min}}$$

MINIMAL DELIVERABLE FIGURES AND GRADING NOTES:

1) Table of constants you used
2) Table of RMSE and NRMSE values for each transfer function and GFP edge propagation plot. Alternatively, values on each plot or figure legend.
3) Steady-state transfer function and model line for each Receiver (GFP vs. [AHL]).
4) GFP edge distance, GFP edge model line, and AHL edge model line over time for each Receiver (mm vs. hours).
5) Figure showing how you generated the distance vs. time plot, including at least one model heat map, one representative image of your plate, and one Receiver.
6) Commented source code in Supplementary.
7) NOTE: The quality of data analysis and interpretation, including clear written explanations for how your model assumptions correlate with how the cells have been genetically programmed, will be weighted more heavily than achieving low error values.

ANALYSIS AND DISCUSSION: These topics should be covered in your final report.

D1. How did you manipulate equations 1-3 to execute your model?

D2. Describe your model design choices (e.g. parameters altered, step size choices), relating them to the design specifications. How did step size impact your compute time and the quality of the approximation of a continuous gradient?

D3. Describe how you defined / measured edge distance in your data or model, and your rationale for the choices.

D4. Does the relative relationship between GFP and AHL edge propagation make sense?

D5. If your system did not work as planned, what do you think went wrong in your implementation and assumptions? What could you do to improve the model, analysis, or experiment in the future?

D6. What can you do in a model that you cannot do in a wetlab experiment?