COURSE MATERIALS

Table of contents

Appendix 1: course syllabus

Course Description

How can we engineer living organisms to develop solutions to serious environmental, medical, or nutritional challenges facing modern world? During the course, students will address this question using the tools of synthetic biology, an interdisciplinary field that leverages concepts from natural sciences and engineering. By programming cells using the genetic code of life, synthetic biologists create organisms that exhibit useful properties, such as identifying and destroying cancer cells, detecting toxins in water, producing low-cost drugs, and manufacturing biofuels. Throughout the course, students will master principles of genetic circuit design, learn about the scope and applications of synthetic biology, and develop their own innovative biological solution to an important real-world problem. Step-by-step guidance in project design will be provided in the form of in-class workshops and homework assignments.

The class will give students a valuable opportunity to improve their independent thinking and communication skills as they collaboratively pursue an open-ended research question. No background in biology is required. In fact, students pursuing all majors are encouraged to join the class because synthetic biology requires collaboration among natural scientists, engineers, social scientists, and humanists.

Course Objectives and Learning Outcomes

Learning goals

- Articulate basic principles of gene expression and regulation
- Interpret abstract representations of genetic circuits, identify their elements and functions, and describe their behavior
- Identify problems in modern society that can be addressed with synthetic biology
- Analyze primary literature to engage with concepts in modern synthetic biology research and identify common strategies for designing a biological system
- Design genetic circuits to perform functions based on desired inputs and outputs of a biological system

Online quizzes (20%)
Students will be asked to complete short online quizzes (posted on Canvas) when readings or videos are assigned as homework. Quizzes should be completed individually. Three attempts will be allowed per quiz and the highest score recorded. Quizzes based on journal articles will assess students' ability to critically read primary literature related to Synthetic Biology and identify main ideas of an article.

Problem-solving assessment (20%)
To assess student mastery of concepts related to the fundamentals of gene expression and interpretation of genetic circuits, students will complete a two-part problem-solving assignment during one class. The first part will consist of a problem set to be solved individually. Following this, students will break into groups to work on a more open-ended question that asks them to predict the behavior of a genetic circuit under different sets of conditions and identify circumstances under which it may fail. The collaborative and individual portions will be weighted equally.

Final Project (50%)

a. Problem proposal (20%)
As a group, students will present a compelling argument that demonstrates they have selected a pressing problem that could be effectively addressed by synthetic biology. They will write a proposal providing an overview of the problem and describing why it is important. It should discuss other attempts to improve the issue (both traditional methods and any related synthetic biology research). After this context building, students should present a preliminary description of how they suggest synthetic biology could be used to address the problem.
Following feedback from instructors, students will have the opportunity to resubmit the proposal. This assignment will help students to prepare their final presentations.

b. Final project presentation (30%)
For the final assignment, students will give a 20 min group presentation discussing their project. Presentation should include background information and explanation of the significance of the problem addressed, genetic circuit design, rationale for choosing the organism into which it will be introduced, and the details of potential real-world implementation of their solution. There will be several in-class workshops to help students develop genetic circuit and prepare an effective presentation.

In-class participation (10%)
Students are expected to attend all classes, take part in discussions based on readings or other homework assignments, meaningfully contribute to group discussions, and complete in-class activities.

Resources

There is no textbook for this class. All required readings will be uploaded to Canvas.
Instructions for accessing additional online material will be provided during the class.

Grade Policies

- Online quizzes 20%
- Problem-solving assessment 20%
- Problem proposal 20%
- Final project presentation 30%
- In-class participation 10%

Absence Policies

Attendance is mandatory. Students who miss more than one class without obtaining instructor permission will not pass the course. Students who have to miss a class due to medical issue, scheduling conflict, or attending a conference should contact the instructors in advance and arrange for making up missed work.

Syllabus Change Policy

This syllabus is only a guide for the course and is subject to change with advanced notice.

Tentative Course Schedule

	In-class Activities	Preparation/Homework due
Part I: Introduction and Background		
Week 1	• Review syllabus and expectations • Introduction to synthetic biology • Group discussion about synthetic biology projects developed for iGEM (International Genetically Engineered Machine)	
Week 2	• Discussion of assigned reading • Abstraction hierarchy in synthetic biology, understanding inputs and outputs of biological systems • Group activity: determining inputs and outputs of an example biological system	• Reading: Matthew Bennett. (2017) "The logic of synthetic biology: turning cells into computers." • Reading: inputs & outputs of biological systems • Online quiz
Week 3	• Basics of gene expression and regulation • Introduction to the standard representation of biological parts • Group activity: combining biological parts to satisfy given prompts	• Reading: types and representation of biological parts • Online quiz
Week4	• Introduction to genetic circuits • Problem solving activity to develop understanding of genetic circuits	• Reading: introduction to genetic circuits • Online quiz
Week 5	• Problem-solving assessment	• Problem set: genetic circuits logic

	In-Class Activities	Preparation/Homework due
Part II: Project Design		
Week 6	• Journal club: discussion of the assigned reading • Strategies for effective reading and analysis of primary literature	• Reading: Pardee, K. *et al*. (2016). Rapid, Low-Cost Detection of Zika Virus Using Programmable Biomolecular Components. • Online quiz
Week 7	• Discussion of areas of interest in synthetic biology ○ Diagnostics, energy, environment**,** food & nutrition, information processing, industrial applications, therapeutics • How can we determine whether a problem can be realistically addressed with synthetic biology?	• Reading: Khalil, A. and Collins, J. (2010). Synthetic Biology applications come of age. • Activity outside of class: students meet with graduate students from Rice BCB and SynBio programs to learn about different research topics in synthetic biology, ask questions about undergraduate research opportunities, etc.
Week 8	• Brainstorming and sharing of real-world problem ideas that students bring to class • Instructors provide feedback on the ideas presented Students each select one problem to develop more fully	• Come up with 3 real-world problems that could be addressed with synthetic biology
Week 9	• Student talks on selected problems • Group formation for the final project, group problem selection	• Choose one of the problems selected in class and prepare a 5-min talk discussing its importance and why it can be addressed with synthetic biology

	In-Class Activities	Preparation/Homework due
Week 10	• Project proposal workshop	
Week 11	• Principles of effective visual communication • Final presentation workshop	• **Problem proposal due midnight before class**
Week 12	• Review circuits designs • Final presentation workshop	• Complete circuit design due Sunday before class. Student will receive feedback via email
Week 13	• **Final project presentations**	• Final presentations due

Appendix 2: Pre-course survey

1. What year are you in?
    - Freshman
    - Sophomore
    - Junior
    - Senior

2. What is your major?

3. Have you taken any college-level biochemistry, bioengineering, or synthetic biology classes? If yes, please list which ones.

4. Have you ever worked on a research project in a lab? If yes, please include the name of the project

5. Why are you interested in taking this class?

6. Please check the boxes next to the topics you are familiar with
    - Central Dogma of molecular biology
    - Regulation of gene expression
    - Genetic circuits
    - Using plasmids for protein expression in bacteria
    - What enzymes are and how they function

7. How comfortable are you with reading peer-reviewed articles related to biology?
    - I have no experience reading peer-reviewed articles
    - I read several articles but feel like they take a lot of effort to understand
    - I am fairly comfortable with reading peer-reviewed articles
    - I have a lot of experience with primary literature

Appendix 3: In-class activities

Week 1. Discussing the examples of synthetic biology projects

Following the course introduction, the students are asked to work in groups and explore several iGEM project examples. The following projects were used in our version of the course:

1. iGEM Peking 2016. Uranium bioremediation. http://2016.igem.org/Team:Peking/Description
2. iGEM Ulberta 2018. Combating Nosema infections in honeybees. http://2018.igem.org/Team:UAlberta/Description
3. iGEM Oxford 2017. Diagnosis of Chagas disease. http://2017.igem.org/Team:Oxford/Description

Students read the project description and answer the following questions:

1. What is the goal of the project? Is there a real-world problem the project is addressing?
2. What is the team's overall approach to solving that problem?
3. What are some of the concepts/terms etc. you struggled to understand (if any)?

Students discuss questions in groups and then present their answers to the rest of the class.

Week 2. Practice questions on abstraction hierarchy and inputs and outputs of a biological system

Part 1: understanding inputs and outputs of biological systems.

1. Microcystin bioremediation
Microcystin is a toxin produced by cyanobacteria that is associated with liver damage if ingested. You are planning to engineer yeast to detect and degrade the toxin in water. Your engineered yeast should be able to detect microcystin and respond by producing yellow fluorescent protein. In addition, once microcystin is detected, yeast should degrade it.
a) Draw a system diagram
b) What devices will you need to build the system? Draw the diagram showing how the devices and how they are connected

2. Zinc biosensor
Zinc is an important micronutrient for human health and its deficiency may result in growth retardation, impaired immune function, and increased mortality risk. To facilitate the diagnosis of zinc deficiency, you are developing a biosensor that detects zinc concentration in human blood serum. Your biosensor will consist of *E. coli* which produces pigment in response to zinc levels. After growing the bacteria in liquid culture, you will mix it with blood serum sample and determine zinc concentration based on fluorescence.
a) Draw a system diagram, showing inputs and outputs
b) Draw the devices you will use for this system
c) What are the advantages of estimating zinc concentration with a biosensor vs. standard laboratory blood test?

The question is based on Watstein, D. M., & Styczynski, M. P. (2018). Development of a Pigment-Based Whole-Cell Zinc Biosensor for Human Serum. *ACS Synthetic Biology*, 7(1), 267–275.

3. Engineering biocontainment
In 2017, Wegman et. al engineered gut bacterium Bacteroides ovatus for drug delivery in humans. This modified bacterium has a thymine auxotrophy (it requires thymine in order to stay alive). Bacteria that escape the digestive tract and lose access to thymine will die, which prevents unwanted propagation of genetically modified microbes in the environment.
a) Draw a system diagram, showing inputs and outputs
b) Draw the devices you will use for this system
c) Can you think of another input that can be used instead of thymine to create a bacterium that dies if it escapes the human body?

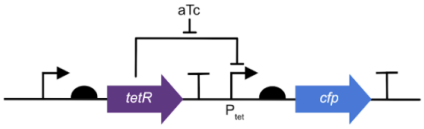
Part 2: Exploring gene expression mechanisms

1. Research how cells can respond to the specified signal and draw a diagram representing this process
Team 1: small molecule (IPTG)
Team 2: light
Team 3: temperature

Hint: you will need to look at promoters and how they respond to each signal.

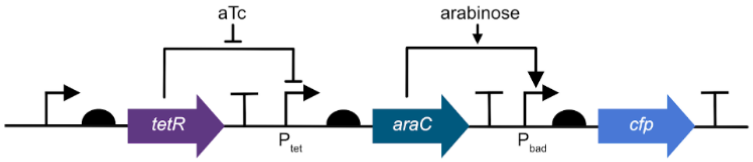
Week 3. Practice problems on gene expression and regulation
During the first part of the class, student teams present their findings on the mechanisms of gene expression and regulation. The remaining time is spent on practice problems which cover the principles of gene expression and regulation.

1. Fill out the truth table for the following genetic circuit:



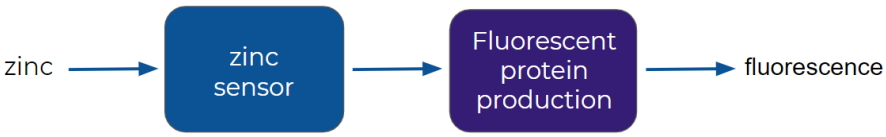
aTc	CFP
0	
1	

2. Fill out the truth table for the following genetic circuit:



aTc	arabinose	CFP
0	0	
0	1	
1	0	
1	1	

3. Create the circuit for zinc biosensor:



You can use the following genetic parts:

- Gene for ZntR repressor which binds to PzntA promoter
- PzntA promoter
- Constitutive promoter
- Gene for RFP reporter
- Two ribosome binding sites (RBS)
- Two terminators

Week 4. Practice problems on genetic circuits
A portion of the class can be spent reviewing the most challenging homework questions.
In-class practice problems:

1. Design a genetic circuit using only repressible and constitutive promoters that has the following behavior:
- produces high levels of GFP when no aTc is added
- produces very low levels of GFP when aTc is added

2. Design a genetic circuit that allows sender cells to produce a signal that activates GPF production in the receiver cells. Sender cells should start producing the signal after you add aTc to the medium.

You can use the following parts:

- Genes: *tetR, gfp, luxI, luxR*

- LuxI: produces AHL
- LuxR: activates Plux promoter when AHL is present
- Promoters: Ptet promoter, Plux promoter, two constitutive promoters
- As many terminators and RBSs as you need

Week 5. Midterm Exam
The midterm exam questions are provided in appendix [].

Week 6. Discussion of a peer-reviewed article

The questions are based on Pardee, K., et al. (2016). Rapid, Low-Cost Detection of Zika Virus Using Programmable Biomolecular Components. Cell, 165(5), 1255–1266.

Part I
1. Figure 2

a. Look at the figures 2A and 2B. Explain how a toehold switch works and its role in Zika virus detection
b. Look at the figures 2C and 2D. Why did the authors test different Zika sensors? What is the difference among them?

2. Figure 3

c. What problem was identified based on the data shown in 3A?
d. How does the technique shown in 3B help solve this problem?

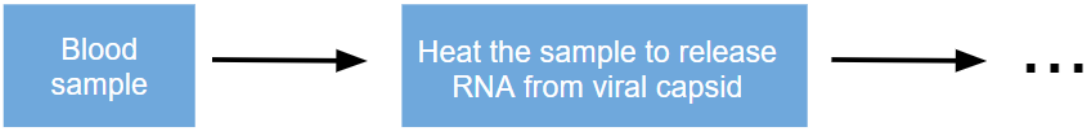
3. Figure 4

e. What conclusions can we make from figures 4A and 4B?
f. Why is the data shown in figures 4A and 4B not yet sufficient to predict that the Zika biosensor will be successful in a clinical setting?
Hint: take a look at Figure 4D

Part II
4. Figure 5

g. Why did the authors want to enable discrimination among Zika strains?
h. What feature distinguishes Zika strains from each other?
i. Explain how CRISPR-Cas9 system was used to distinguish viral strains. What is the role of PAM?

Part III
5. Starting with a blood sample from macaque infected with Zika, draw a diagram showing the full diagnostic workflow



Week 7. Discussion of areas of interest in synthetic biology

1. After the discussion of homework questions, the students review iGEM projects from different competition tracks.
Each team selects a project from a selected track: http://2018.igem.org/Competition/Tracks/
The teams answer the following questions:

- Describe the problem the project is addressing
- What approach did the team use to solve the problem? Draw a system-level representation, if appropriate
- Describe the genetic circuit(s) created in the project. What synthetic biology tools were used (ex. repressors/activators, CRISPR, RNA regulation, etc.)
- Select one primary article the project cites. Go to that article - can you think of some ways it inspired the project idea/genetic circuit/methods?
- Identify at least two limitations of this project or issues that could be encountered with its implementation

2. To prepare for the upcoming brainstorming assignment, in which students are asked to come up with problems that can be addressed with synthetic biology, the class discusses good and bad examples of different problem statements:

Bad problem statement	Good problem statement
Cancer is a problem	Lack of specific and controlled treatment options for cancer that allow killing mechanism to be localized in the tumor and reduce side-effects (iGEM Zurich 2017). *Ideally, you would also identify a specific type of cancer*
Plastic pollution	Polyethyleneterephthalate (PET) is widely used in different applications such as electronics and textile industry. It is very difficult to degrade, which poses an environmental problem (iGEM TJUSLS 2016).
Food waste	Ethylene is a plant hormone regulating fruit ripeness. Lack of control over ethylene levels creates a situation where large percentage of fruits become spoiled before they are delivered to a consumer (iGEM USYD 2016).

Week 8. Project ideas brainstorming

The full class period is used to discuss student ideas. Each student selects one idea to do more research on and prepare a 5-min presentation for the next class. The presentations are graded based on how well the student covers the following points:

- General description of the project idea
- Brief overview of previous similar projects/papers

- Explanation of the proposed approach, including inputs and outputs for the proposed biological system and synthetic biology tools that can be used
- Feasibility concerns

Week 9. Student presentations on selected project ideas
Each student gives a presentation on the selected project idea. The teams discuss the idea and each selects one of the ideas for creating a project proposal.

Week 10. Project proposal workshop
The goal of the class is for the teams to receive feedback on their project proposals. To do this, the teams exchange their papers on Google Drive, add comments, and write a separate document to summarize the comments. The remaining time is allocated for the teams to work on refining their project proposal, drafting genetic circuits, and working on the final presentation.

Week 11. Scientific communication workshop
The class discusses tips for creating professional scientific presentations. The main suggestions include:
1. Emphasizing visual elements (figures, plots, diagrams, tables) over text paragraphs
2. Using descriptive titles that help the audience understand the main take-home points of each slide
3. Including animation where it can be used to improve the flow of the presentation
4. Including clear outlines, goal statements, and summaries

The remaining time is allocated for students to work on the final presentation, ask questions related to project design, and receive feedback on drafts.

Week 12. Final presentation workshop
The full class is allocated on finishing the presentations and discussing any remaining questions.

Appendix 4: Midterm exam

Exam

Question 1

Order the following steps to accurately describe the process of protein expression (3 pts)

A. Peptide folding
B. Ribosome binds to RBS
C. Ribosome encounters stop codon
D. RNA polymerase binds to promoter
E. Transcription elongation
F. Translation elongation

Underline the part of the process that involves complementary base pairing and draw a box around the part of the process that uses the codon triplet code. (1 pt)

DNA → **→ protein**

Question 2 (6 pts)

Based on iGEM ETH Zurich 2017 project

You want to design a bacterium that detects and kills cancer cells. To prevent engineered bacteria from targeting healthy tissues, you decide that the killing mechanism will only be activated under the following conditions:

Condition 1: High density of bacteria **and** high lactate concentration

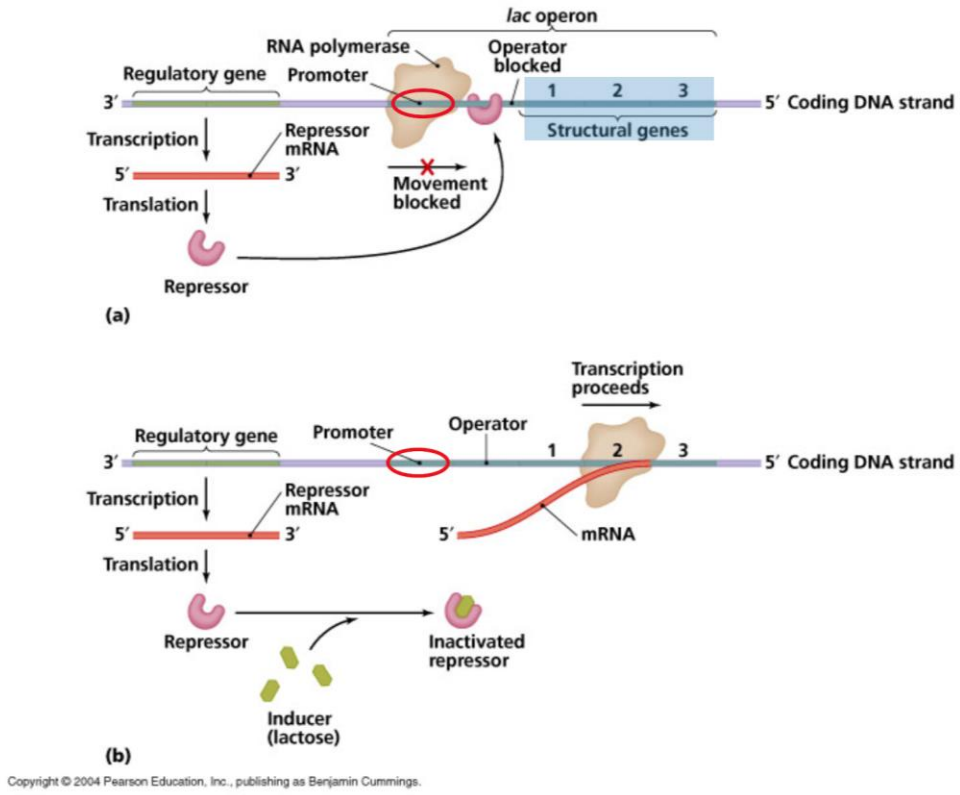
Condition 2: Temperature is increased from 37°C to 45°C

Condition 1 is associated with cancer markers while Condition 2 is introduced so that killing is only activated when focused ultrasound is applied to a particular area of the body, which causes an increase in temperature.

When both conditions are fulfilled, the bacterium bursts and releases a toxin that targets cancer cells. **The production of toxin starts when the condition 1 is true but its release only occurs when <u>both</u> conditions are fulfilled.**

1) Draw a diagram of this system showing inputs and outputs
2) Draw a device-level representation of this system

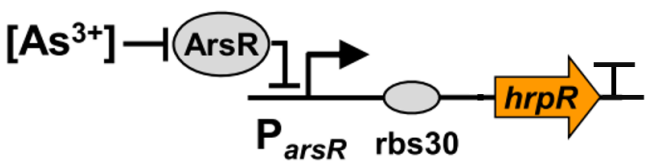
Question 3 (9 pts)



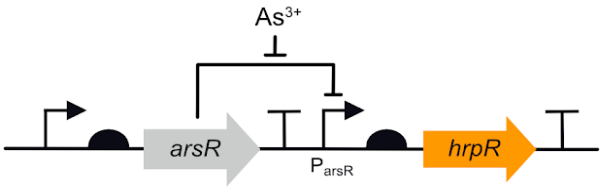
a) Is the circled promoter constitutive or regulated? Explain your answer
b) The repressor shown in the figure is LacI. If we expressed TetR instead of LacI, keeping everything else the same, would it be able to repress the transcription of structural genes (highlighted in blue)? Explain your answer.
c) Draw a genetic circuit based on this diagram using standard symbols for biological parts that we covered in class.

Question 4 (6 pts)
This question is based on Wang, B., Barahona, M., and Buck, M. (2013). A modular cell-based biosensor using engineered genetic logic circuits to detect and integrate multiple environmental signals *Biosensors and Bioelectronics,* 40(1): 368–376

a) Fill out the truth table for the following genetic circuit (2 pts):

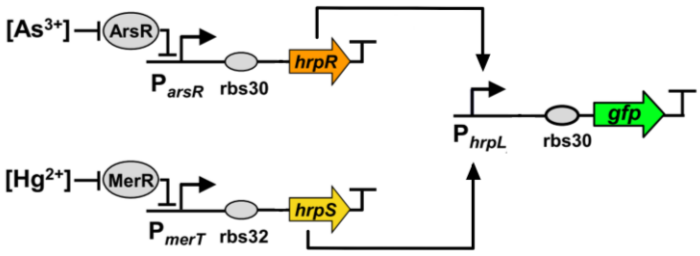


Note that in a more familiar representation, the circuit looks as follows:



As^{3+}	HrpR
0	
1	

b) HrpR and HrpS are two activators for the PhrpL promoter and the presence of both is required to initiate transcription from this promoter. Fill out the truth table for the following genetic circuit: (4 pts)



As^{3+}	Hg^{2+}	GFP
0	0	
0	1	
1	0	
1	1	

c) What logical operation is this circuit performing? (1 pt)

Appendix 6: Project proposal assignment

Instructions

Communicate with your team to select one project idea that you will be moving forward with. The options for choosing an idea are:

1) Choose one from those your team members presented on
2) Choose one from another team, as long as they are not using it
3) You *can* come up with a completely new idea, in which case you will need to contact the instructors in advance to get approval.

The next step in the project design process is to develop a more detailed proposal which investigates different aspects of this problem and outlines a preliminary solution. This assignment will help you build background knowledge related to you project idea and collect information that you will use in your final presentation.

Your proposal should address the following points:

- Description of the problem and explanation of its importance **(5 pts)**
- Overview of previous attempts to solve this problem (both biological and non-biological) **(10 pts)**
- Explanation of why synthetic biology can provide a more effective solution **(5 pts)**
- A preliminary idea of a biological system that can be designed to address this problem **(50 pts)**
    - Describe the general desired behavior and properties of the biological system
    - What inputs and outputs would it have?
    - Draw a device-level representation of your system
    - Come up with a genetic circuit draft
- Potential challenges you may encounter in designing/building/implementing your synthetic biology solution **(10 pts)**

You should work on this assignment as a team and submit one document that you write together.

You can use any online or other resources to complete this assignment. You are encouraged to contact instructors and graduate students for help.

Your work should reference valid sources, including but not limited to primary literature and other peer-reviewed articles **(20 pts)**

Formatting

- Length: 2-3 pages, double-spaced
- Font: 12 pt, Times New Roman

Total points possible: 100 pts

Appendix 7: Final presentation instructions

Please prepare a 15-minute presentation describing your proposed project.

Your presentation should address the following topics:

- Description of the problem and explanation of its importance
- Explanation of why synthetic biology can provide a more effective solution compared to previous ones
- Approach: a detailed description of biological system that can be designed to address this problem (you should spend the most time on this portion of the presentation)
- Describe the desired behavior and properties of the biological system
- What inputs and outputs would it have?
- Propose a genetic circuit: you should include what specific genes you will use and how regulation will be achieved
- Briefly but specifically describe what experiments you would perform to test your system
- Potential challenges you may encounter in designing/building/implementing your synthetic biology solution

Following your presentation there will be 5 minutes for Q&A.

Appendix 8: Sample student project proposal

Improving crop climate-resilience using engineered rhizospheres

Current worldwide trends suggest that humanity is having difficulty sustaining itself with crop production. Such a trend arises from the sheer difficulty of using crops as a means of sustenance; drought, harsh winters, and other environmental factors make it difficult for crops to yield reliable harvests. There has been considerable interest in engineering crops for various resistances, but genetic engineering is usually done through germline editing which is inefficient and time consuming (Arora, 2017). As such, it is beneficial to consider genetically engineering the rhizosphere of plants rather than the plants themselves for the sake of efficiency and public perception.

Given that reliable harvests are difficult enough for the earth's growing population, climate change and increasing temperatures will undoubtedly affect the growing conditions for many crops, specifically cacao trees. Cacao trees thrive in rich, well-drained soils in the tropics, but increasing global temperatures and drought conditions are projected to increase cacao tree mortality and decrease production in the coming years (Schmitz, 2015). Additionally, droughts increase the infection rates of fungal diseases in cacao plants (Gateau-Rey et al., 2018), which worsen the problem for cacao tree growth. The importance of improving the climate resilience of the cacao plants is essential because of its potential applications to other crops to increase crop yield and productivity.

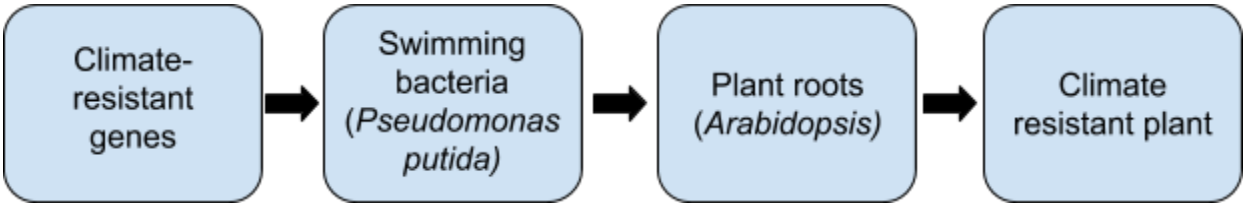
Previous solutions include changes in resource and crop allocation which simply pushes off the problem as opposed to providing a permanent solution (Howden et al., 2007). This same study claims that production diversification will help combat multiple other issues such as climate variability. However, another pitfall of this solution is that burgeoning populations may not be able to reduce their crop intake to the levels suggested by the study. As a result, a study in which the biological component of this issue may provide a long term solution to the issue.

Current methods that try to mitigate the effects of drought stress are not very effective or require genetic modification of the germline. For example, chemicals have been used to increase the function of abscisic acid (ABA), which closes plant stomata and reduces water loss to increase drought resistance. Unfortunately, the limitations of ABA are its chemical instability and fast catabolism. Moreover, other methods have focused on the overexpression of pyrabactin resistance 1/PYR1-like/regulatory components of ABA receptor (PYLs) in *Arabidopsis* to increase their resistance to drought stress (Cao, 2017). However, these methods can result in developmental defects and decreased yield. Synthetic biology can provide a more effective and cheaper solution that does not require copious amounts of chemicals or genetic engineering of the germline itself.

In order to confer increased climate resistance, our group intends to genetically engineer the rhizosphere of crops. Specifically, our project would engineer soil bacteria, *Pseudomonas putida*, that have the ability to undergo horizontal gene transfer for the sake of transferring climate resistance related genes. The genes that will be used will be the gene for drought resistance helping protein: ACBP2 (Du, 2013). Additionally, the genes for CspA and CspB will be used as they help with cold weather and other general climate related stresses (Sanghera 2011). Ideally, these bacteria would horizontally transfer genes to their host plants, which would be various cash crops that have an inability to survive under harsh conditions.

Additionally, these bacteria would have the ability to swim towards roots to then induce horizontal gene transfer of increased climate resistance genes. Inputs would likely be the presence of some sort of chemical in the soil (testing needed) such as and the output would be the swimming of the bacteria to the plants. Then, the input would induce the output of horizontal gene transfer. Such a solution is better than previous solutions in that it is more efficient and does not require independent germline editing. Additionally, the applicability of bacteria with such horizontal gene transfer capabilities is very high in that they can be dispersed into soil in high amounts that can be transported and packaged or they can be inserted into pinpoint locations in soil through some type of drip mechanism.

Potential challenges we may face in implementing our synthetic biology solution are that cacao trees are difficult to grow in a lab setting, but to overcome this problem, we intend to use *Arabidopsis thaliana* since it is a model genetic organism.



Citations

Arora, L., & Narula, A. (2017). Gene Editing and Crop Improvement Using CRISPR-Cas9 System. *Frontiers in plant science*, 8, 1932.

Cao, M., Zhang, Y., Liu, X., Huang, H., Zhou, X. E., Wang, W., Zhu, J. (2017, October 30). Combining chemical and genetic approaches to increase drought resistance in plants. Retrieved from https://www.nature.com/articles/s41467-017-01239-3

Du, Z. et al.. (2013), Overexpression of Arabidopsis acyl-CoA-binding protein ACBP2 enhances drought tolerance. *Plant, Cell & Environment*, 36: 300-314.

Eckardt, N. A., Cominelli, E., Galbiati, M., & Tonelli, C. (2009). The future of science: food and water for life. *The Plant Cell*, 21(2), 368-72.

Gateau-Rey L, Tanner EVJ, Rapidel B, Marelli J-P, Royaert S (2018) Climate change could threaten cocoa production: Effects of 2015-16 El Niño-related drought on cocoa agroforests in Bahia, Brazil. *PLoS ONE*. 13(7).

Howden, Mark, et al. "Adapting Agriculture to Climate Change." *PNAS*, vol. 104, no. 50, 11 Dec. 2007, pp. 19691–19696.

Sanghera, G. S., Wani, S. H., Hussain, W., & Singh, N. B. (2011). Engineering cold stress tolerance in crop plants. *Current genomics*, 12(1), 30-43.

Schmitz, Harold. "The Race to Save Chocolate." *Scientific American*, 1 June 2015.

Appendix 10. Readings prepared by the instructors

I. Abstraction hierarchy in synthetic biology

Last class, one of the iGEM projects we discussed involved engineering bacteria to diagnose Chagas disease. The details of the genetic circuit used in the project were rather complicated, but we were able to summarize the approach with the following diagram:

Figure 3. A simple diagram describing Chagas disease biosensor.

By drawing this diagram, we created an *abstraction* of the system which describes how it works in simple terms. Abstraction is a useful concept because it allows us to approach design or understanding of biological systems without dealing with all the complex details at the same time. To this end, synthetic biologists came up with an abstraction hierarchy, where information describing biological functions can be organized across several levels of complexity (Figure 2).[1]

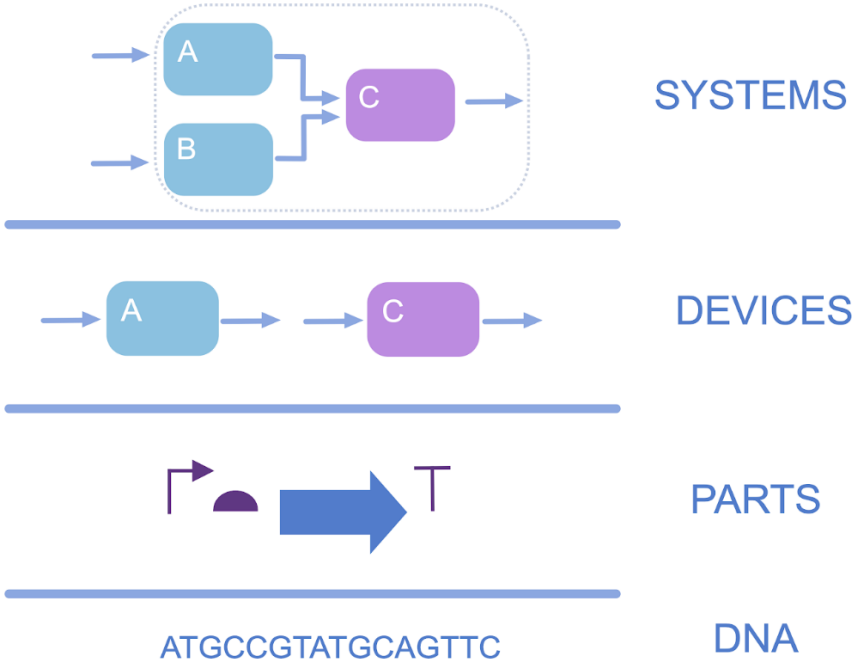


Figure 2. Abstraction hierarchy in synthetic biology. Adapted from Endy (2005).

At the top, we have a system-level representation which shows the inputs and outputs of the system. In

other words, it tells us what the system does in response to external signals. On a more detailed level, systems consist of devices representing specific biological functions. Devices are made up of parts (pieces of DNA with distinct features which encode for the desired functions). Finally, at the very bottom, we have DNA – the exact sequence of nucleotides we would use for each part.

To better understand the abstraction hierarchy, let's consider an example. Imagine you want *E. coli* to produce green fluorescent protein (GFP) if it senses high concentration of arsenic in water. This would allow you to determine whether water is contaminated by adding bacteria and then measuring the fluorescence signal. The system-level representation will look like this:

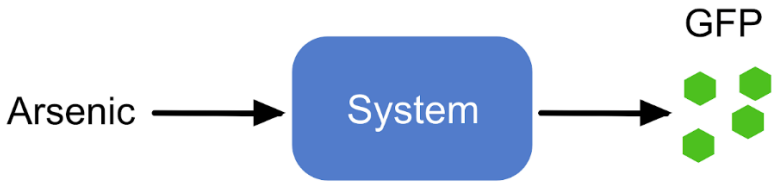


Figure 3. Arsenic biosensor (system-level)

Biological functions we are interested in include the detection of arsenic and GFP production, so the device-level representation will be as follows:

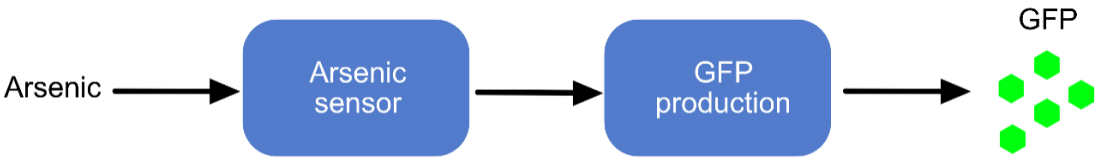


Figure 4. Arsenic biosensor (device-level)

Parts will include pieces of DNA required for each device. We are going to discuss parts later in the course.

Inputs and outputs of biological systems

As you saw above, if we want to build a biological system, one approach is to start by identifying its inputs and outputs. The inputs are environmental signals while the outputs are the behaviors we are interested to achieve or products we want to make. Read the excerpt adapted from Wang (2013)[2] below to learn more about inputs and outputs. Please look up any words you are not familiar with.

Excerpt from Wang (2013)

"Bacterial cells live in an ever-changing environment and must therefore be equipped with specific genetically-encoded sensors and signaling networks to continuously perceive and react to the various environmental signals. Analogous to a typical electromechanical sensor, a cellular signaling network normally consists of three interconnected modules – the input sensors, internal processing and regulatory circuits and output actuators to allow signal sensing and timely adaptations in cell physiology.

The input sensors are receptors, either embedded in the cell membrane (e.g. sensor kinases) or located freely in the cytoplasm (e.g. ligand responsive allosteric proteins) with which the cell can detect various extra- or intra-cellular signals, such as chemical molecules, metal ions, light, heat or antigens, and

transduce them into differential gene transcriptional levels. Downstream gene regulatory networks process and integrate such signals combinatorially for a logic decision to be made, mimicking the digital logic circuit in electronic circuitry. Decisions are signified by changes in the expression of output actuators: the relevant proteins and chemicals responsible for the final phenotypic changes in motility, growth and morphology etc.

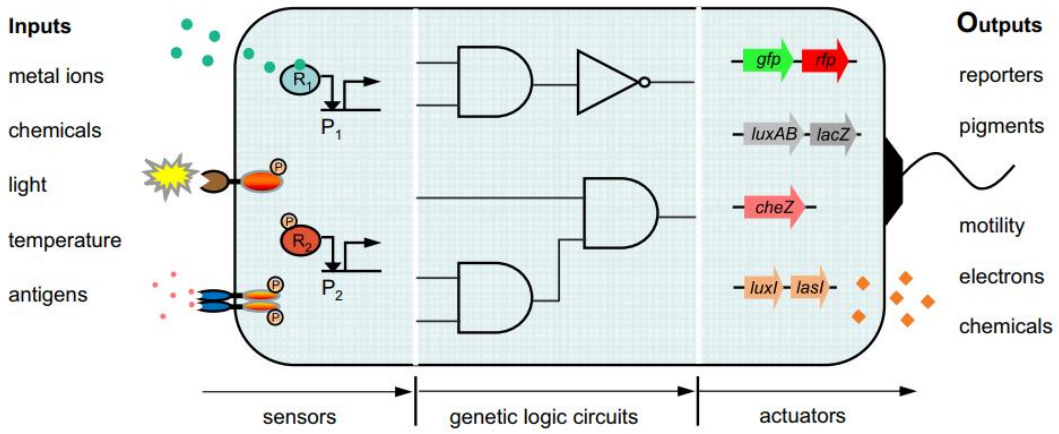


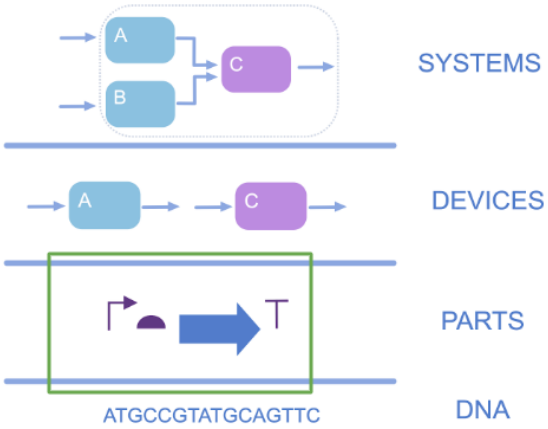
Figure 5. common inputs and outputs of a biological system. Wang et al. (2013)

Bacterial cells can be viewed as programmable living biosensors in which the three component modules are exchangeable (Fig. 1). For example, specific synthetic sensors can be developed from either the host's own genetic repertoire or that of other bacterial species with more relevant specializations in sensing capabilities in order to detect, for example, particular environmental contaminants or disease-related signals. Informed by the advanced sensing capabilities of many environmental microbes, already a number of single-input bacterial biosensors have been constructed to detect various toxic pollutant such as arsenic xylene and toluene (DNT explosive) and the human pathogen *Pseudomonas aeruginosa* with fluorescence, luminescence or colorimetric pigments as outputs."

1. Endy, D. (2005). Foundations for engineering biology. *Nature*, *438*(7067), 449–453.
2. Wang, B., Barahona, M., Buck, M. (2013). A modular cell-based biosensor using engineered genetic logic circuits to detect and integrate multiple environmental signals. *Biosensors and Bioelectronic*s, *40*(1), 368-376.

II. Types and representation of biological parts

Last class, we introduced the abstraction hierarchy in synthetic biology and discussed systems and devices. We will now move on to the next level and talk about parts.



According to Canton (2008), a biological part is "a genetically encoded object that performs a biological function and that has been engineered to meet specified design or performance requirements." [1] In other words, a part is a sequence of DNA which encodes for a distinct biological function and plays a specific role in gene expression.

Read the following article to review the steps of gene expression: https://www-nature-com.ezproxy.rice.edu/scitable/topicpage/translation-dna-to-mrna-to-protein-393

Four biological parts essential for gene expression are shown in the table below:

Part	Function	Symbol
Promoter	A sequence of DNA where RNA polymerase binds and initiates transcription	
Ribosome binding site (RBS)	A sequence of mRNA where ribosome binds and initiates translation	
Coding sequence (CDS)	A sequence of DNA which encodes for a protein	

Terminator	A sequence of DNA which causes RNA polymerase to dissociate and stop the transcription	

The symbols representing biological parts are specified by the Synthetic Biology Open Language (SBOL). The aim of SBOL is to create a set of standardized symbols and produce a coherent language that can be used to easily communicate synthetic biology designs in diagrams.

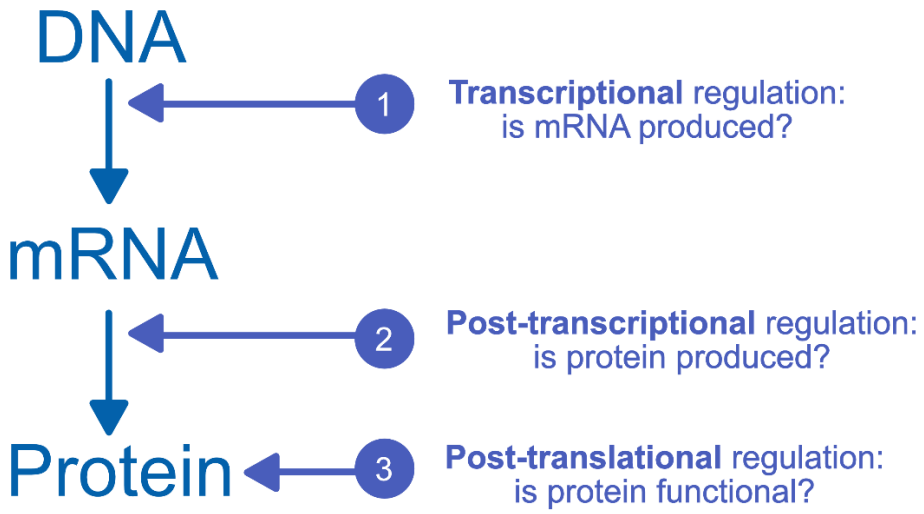
Question 4. Go to http://sbolstandard.org/visual/ and read the description of SBOL. Next, go to http://sbolstandard.org/visual/glyphs/ and scroll to "Nucleic acid glyphs." Select 3 glyphs that you find interesting and research the biological functions they represent using any online resources. Write what you found in your answer.

For example, if you want to create a system for the expression of green fluorescent protein (GFP) in *E. coli*, you can take the parts from the table and create the following genetic circuit:

Question 5. You ordered a (linear) piece of DNA containing the genetic circuit for GFP expression (shown above) from IDT DNA synthesis company. Briefly describe what steps you will take to make E. coli produce GFP using your genetic circuit. How would you confirm that your strategy was successful? You can use any online resources to answer this question. Multiple correct answers are possible.

Gene regulation

Reliable control of gene expression is critical for building genetic circuits that can respond to different inputs and perform the desired functions. Gene expression regulation can be implemented on three different levels: transcriptional, post-transcriptional, and post-translational.



Transcriptional regulation

Watch this video before moving on: https://www.youtube.com/watch?v=3S3ZOmleAj0

Transcriptional regulation is all about promoters, which control the binding of RNA polymerase and transcription factors. Since the promoter region drives transcription of a target gene, it therefore determines the timing of gene expression and largely defines the amount of protein that will be produced. Many common promoters are always active and thus referred to as **constitutive promoters**. Others are only active under specific circumstances. They are called **inducible promoters**, and can be switched from an **OFF** to an **ON** state.[3]

Question 6. Research the mechanism of how a given signal can be used to turn the transcription on or off (only do this for one signal that your team started researching in class). Some helpful links are provided below but you can use any other online resources if you want.

Now imagine that you want to use this signal to turn GFP expression either on or off (choose one) in the following genetic circuit:

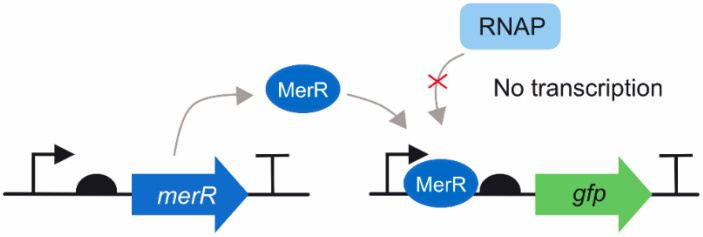
What promoter will you use? What additional proteins will need to be expressed? Draw a diagram showing how the presence of the signal will lead to GFP expression. You can sketch it by hand and upload a picture.
See the figure on the next page for a diagram example.

Team 1: small molecule (IPTG)
https://www.biologicscorp.com/blog/iptg-induction-protein-expression/#.XEFqOlxKiUk
http://agscientific.com/blog/2016/01/iptg-in-synthetic-biology-top-3-review-papers/

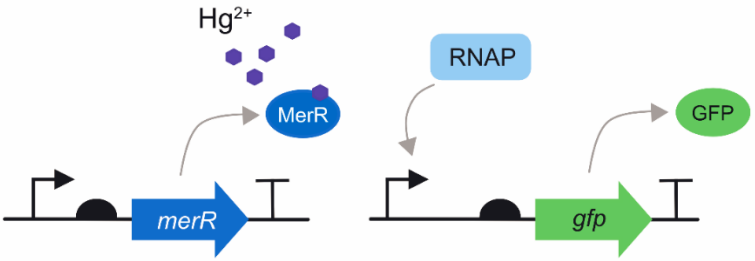
Team 2: light
https://www-nature-com.ezproxy.rice.edu/articles/nature04405
https://www-ncbi-nlm-nih-gov.ezproxy.rice.edu/pmc/articles/PMC3424386/

Team 3: temperature
http://parts.igem.org/Part:BBa_K200011
https://www-ncbi-nlm-nih-gov.ezproxy.rice.edu/pmc/articles/PMC2848208/

Example: turning ON GFP expression with metal ion[4]



No Hg: repressor binds to the promoter region, blocking RNA polymerase access; transcription does not occur.



Hg added: Hg inactivates the repressor and prevents it from binding the promoter region. RNAP can freely acces the promoter and begin transcription.

References

1. Canton, B., Labno, A., & Endy, D. (2008). Refinement and standardization of synthetic biological parts and devices. *Nature Biotechnology*, *26*(7), 787–793.

2. Synthetic Biology open language. http://sbolstandard.org/visual/glyphs/

3. Adapted from https://blog.addgene.org/plasmids-101-repressible-promoters

4. Adapted from http://2014.igem.org/Team:UFAM_Brazil/Biosensor