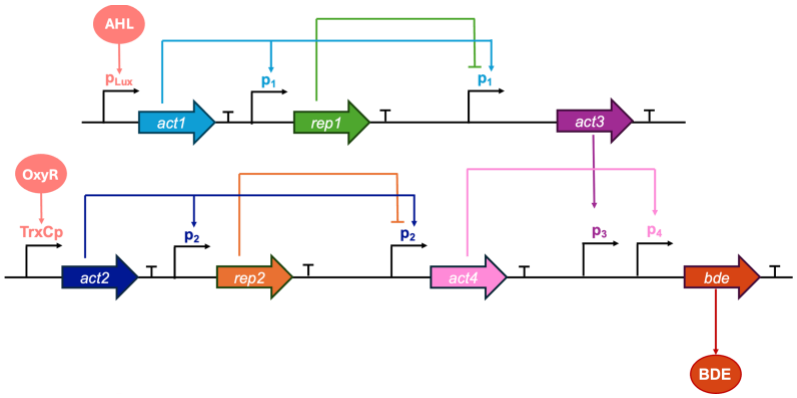
Dual-Input Pulsed Interval Circuit for Biofilm Disruption

Developing a method to specifically detect and disrupt biofilms could have transformative implications for healthcare by reducing infection rates, treatment costs, and patient suffering.[1] This project aims to inform the design of systems capable of detecting two specific signals associated with biofilm formation – N-acyl homoserine lactone (AHL) and oxidative stress (OS) – and, in response, produce a transient pulse of enzymes to disrupt biofilm development.[2] This system is designed to function only when both signals are present, ensuring targeted action against biofilms while minimizing unintended effects of the surrounding environment.

To achieve this selective response, the system employs two separate incoherent feedforward loops (IFFLs), one for each input signal. Incoherent feedforward loops are a well-characterized network motif in both natural and synthetic biological systems, known for their ability to produce pulse-like responses to sustained stimuli.[3] An IFFL occurs when an initial signal activates both a target gene and a repressor of that gene, leading to an initial surge in the target gene's expression followed by repression.



1. **AHL Detection Pathway:**

When external AHL is present due to biofilm formation by other bacteria, it diffuses into the cell and binds to LuxR, forming the LuxR-AHL complex. The LuxR – AHL complex activates the production of *act1*, an activator protein downstream of pLux.[4] Activator 1 activates the expression of Repressor 1. Activator 1 also activates Activator 3 production; however, Repressor 1 represses Activator 3 expression. This creates an incoherent feedforward loop where Activator 1 both promotes and, through Repressor 1, indirectly inhibits Activator 3 production. The IFFL results in a transient pulse of Activator 3 expression, crucial for temporal control of the downstream response.

1. $\frac{d[AHL]}{dt} = \alpha_{AHL} - \delta_{AHL} \cdot [AHL]$
2. $\frac{d[Act1]}{dt} = \alpha_{Act1} \cdot \frac{[AHL]^{n_{AHL}}}{K_{AHL}^{n_{AHL}} + [AHL]^{n_{AHL}}} - \delta_{Act1} \cdot [Act1]$

[1] Hall-Stoodley, Costerton, and Stoodley, "Bacterial Biofilms."
[2] Wu et al., "Role of Oxidative Stress in Persister Tolerance."
[3] Goentoro et al., "The Incoherent Feedforward Loop Can Provide Fold-Change Detection in Gene Regulation."
[4] Basu et al., "A Synthetic Multicellular System for Programmed Pattern Formation."

3. $$\frac{d[Rep1]}{dt} = \alpha_{Rep1} \cdot \frac{[Act1]^{n_{Act1}}}{K_{Act1}^{n_{Act1}} + [Act1]^{n_{Act1}}} - \delta_{Rep1}[Rep1]$$

4. $$\frac{d[Act3]}{dt} = \alpha_{Act3} \cdot \frac{[Act1]^{n_{Act1}}}{K_{Act1}^{n_{Act1}} + [Act1]^{n_{Act1}}} \cdot \frac{K_{Rep1}^{n_{Rep1}}}{K_{Rep1}^{n_{Rep1}} + [Rep1]^{n_{Rep1}}} - \delta_{Act3} \cdot [Act3]$$

2. Oxidative Stress Detection Pathway:

Under oxidative stress, OxyR protein becomes oxidized which activates its ability to transcribe genes responsible for defense mechanisms. Activator 2 is downstream of an OxyR-activated promoter, TrxCp (Part:BBa_K1104201), so low oxygen conditions, such as within a biofilm, activate the expression.[5] Activator 2 then promotes the expression of Activator 4, which is also downstream of TrxCp. Repressor 2 represses Activator 3 expression by binding to an upstream promoter. Like the first pathway, this forms an IFFL where OxyR activates Activator 2 which both activates and, through Repressor 2, indirectly inhibits Activator 4 production. The IFFL results in a transient pulse of Activator 4 expression, aligning the system's response with the presence of oxidative stress.

5. $$\frac{d[OxyR]}{dt} = \alpha_{OxyR} - \delta_{OxyR} \cdot [OxyR]$$

6. $$\frac{d[Act2]}{dt} = \alpha_{Act2} \cdot \frac{[OxyR]^{n_{OxyR}}}{K_{OxyR}^{n_{OxyR}} + [OxyR]^{n_{OxyR}}} - \delta_{Act2} \cdot [Act2]$$

7. $$\frac{d[Rep2]}{dt} = \alpha_{Rep2} \cdot \frac{[Act2]^{n_{Act2}}}{K_{Act2}^{n_{Act2}} + [Act2]^{n_{Act2}}} - \delta_{Rep2} \cdot [Rep2]$$

8. $$\frac{d[Act4]}{dt} = \alpha_{Act4} \cdot \frac{[Act2]^{n_{Act2}}}{K_{Act2}^{n_{Act2}} + [Act2]^{n_{Act2}}} \cdot \frac{K_{Rep2}^{n_{Rep2}}}{K_{Rep2}^{n_{Rep2}} + [Rep2]^{n_{Rep2}}} - \delta_{Act4} \cdot [Act4]$$

3. Integrated IFFLs for Biofilm Disrupting Enzyme Production

The signals are integrated so that the production of the biofilm-disrupting enzymes (BDE) requires high levels of both Activators 3 and 4. Since Activators 3 and 4 are produced transiently and only in response to their respective signals, the enzymes are synthesized only when both AHL and oxidative stress are present. Therefore, the system exhibits AND gate behavior. The use of two IFFLs ensures that enzyme production is tightly regulated in time, producing a pulse of enzyme activity that is sufficient to disrupt biofilm formation without continuous expression.

9. $$\frac{d[BDE]}{dt} = \alpha_{BDE} \cdot \frac{[Act3]^{n_{Act3}}}{K_{Act3}^{n_{Act3}} + [Act2]^{n_{Act3}}} \cdot \frac{[Act4]^{n_{Act4}}}{K_{Act4}^{n_{Act4}} + [Act4]^{n_{Act4}}} - \delta_{BDE} \cdot [BDE]$$

General Assumptions:

This model focuses on the dynamics of an integrated incoherent feedforward loop using endogenous promoters. For simplicity, this model assumes that:

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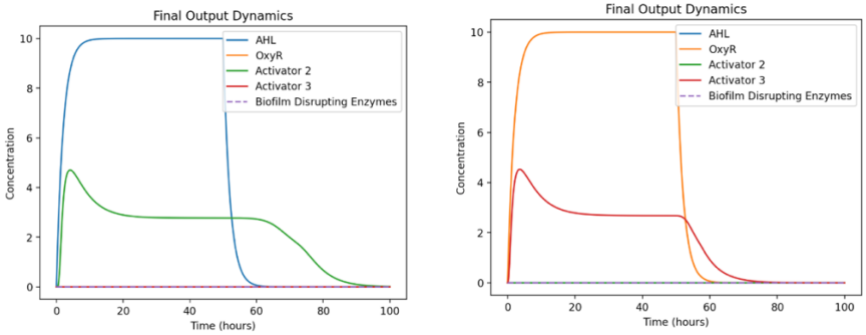
[5] Pomposiello and Demple, "Redox-Operated Genetic Switches"

1. mRNA dynamics can be neglected, and protein production is directly linked to gene activation without significant delays. Basal production is incorporated into the production rate.
2. There is no crosstalk or unintended interactions between the AHL detection IFFL and the oxidative stress detection IFFL. Each IFFL operates independently until they converge at the point of enzyme production.
3. The activation and dimerization of OxyR as a response to oxidative stress can be represented as an increase in the species OxyR. Low oxygen leads to its accumulation and subsequent activation of downstream genes. This is also a simplification of the oxidative stress response by modeling stress as a single parameter (OxyR), rather than modeling the whole signaling pathway.
4. The presence or absence of external AHL is modeled by adjusting the production rate parameter α_{AHL} within the cell. Instead of modeling AHL diffusion and transport explicitly, the model adjusts α_{AHL} to simulate varying AHL input functions. This is also a simplification of quorum sensing by reducing the system to only one of the molecules pathways rather than modeling the whole signaling pathway.
5. These circuits are activated by the endogenous biofilm formation response. This is assumed to use already existing resources in the cell, including the preformed LuxR-AHL complex and activated OxyR ready for transcription in enough excess that it can trigger the circuits while maintaining the viability of the cell. Furthermore, it assumes sufficient resources in the cell to perform these functions.
6. α, the production term, can be used to represent promoter strength and δ, the degradation term, can be used to represent regulator stability.

Analysis

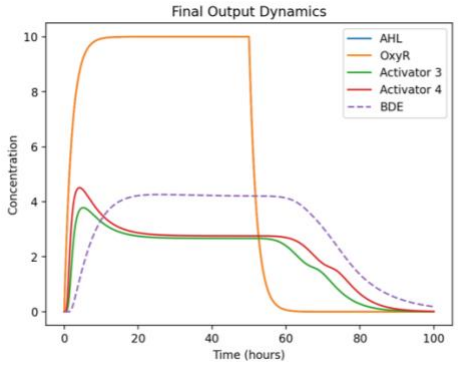
The dynamics between the two IFFLs determine the BDE output of the system. First, I isolated each signal (AHL and OxyR) to evaluate the independent functionality of the quorum sensing and oxidative stress modules. The results confirmed that without both input presents, there is no production of biofilm disrupting enzymes.

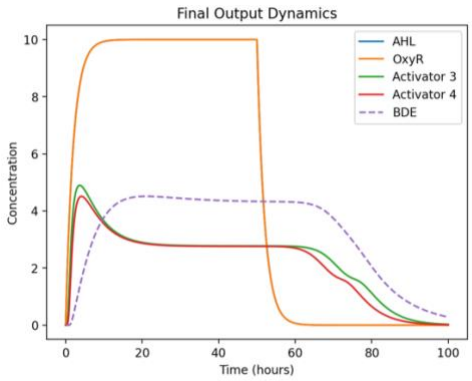
Figure 1 – Single Input Testing of BDE Output. AHL-only input and OxyR-only input.



In the video presentation, I use the simulation tool to explore how promoter and regulator choice impact production of BDEs. Promoter strength, represented as production rate, and regulator stability, represented as degradation rate, affect the timing and overlap of activator pulses in the circuit.

Figure 2 – Increasing Promoter Strength: α_{Act1} = 3.0 → 10.0 (increases peak concentration and pulse duration)



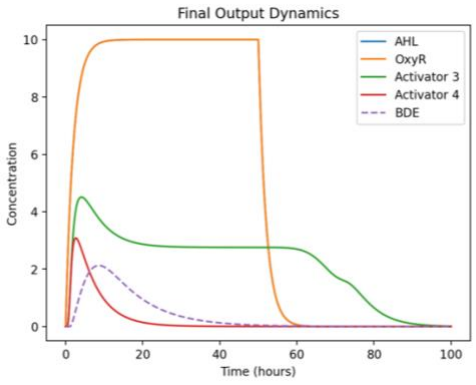


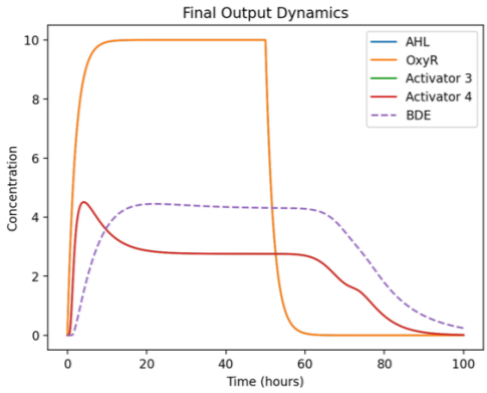
Peak BDE Concentration: 4.37
Time to Peak BDE: 23.73 hours
Pulse Duration: 76.94 hours

Peak BDE Concentration: 4.51
Time to Peak BDE: 21.06 hours
Pulse Duration: 73.49 hours

For my first experiment, I investigated how varying the strength of promoter 1 affects the dynamics of downstream species. This is modeled through the production rate of Activator 1. With a low strength promoter, Activator 1 has a lower peak height and shorter pulse duration. This, in turn, reduced the activity of downstream species such as Repressor 1 and Activator 3. However, as the promoter gets stronger Activator 1's peak increases. This causes the BDE peak to also increase and the pulse duration to be prolonged, however one the Activator 3 response matches Activator 4's, the BDE output is limited by Activator 4 from the OS module.

Figure 3 – Increasing Regulator Stability: δ_{Rep2} = 0.05 → 0.5 (decreases peak concentration and pulse duration)





Peak BDE Concentration: 2.13
Time to Peak BDE: 8.62 hours
Pulse Duration: 15.16 hours

Peak BDE Concentration: 4.45
Time to Peak BDE: 22.14 hours
Pulse Duration: 71.99 hours

For my next experiment, I investigate how varying the degradation rate of a repressor affects BDE production. A lower degradation rate of Repressor 2 translates to a longer lifespan in the circuit. For simplicity, I did not consider multiple binding and unbinding events for each produced protein. Since Repressor 2 persists longer, there is a prolonged suppression of Activator 4. As the degradation rate of Repressor 2 increases, Activator 4 can escape suppression sooner. This leads to earlier and higher peaks in BDE concentration, with increased pulse duration and total enzyme output.

This system and these experiments begin to describe how changes in promoter strength or regulator stability, changes often made when designing and engineering systems, can influence the amplitude and duration of BDE production from the integrated IFFLs.

Interactive Simulation Instructions:

1. Download attached file (final_project_sim.py)
2. Run from Terminal (streamlit run final_project_sim.py)

Bibliography

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