Resisting Resistance: *A Synthetic Biology Approach to Counter Antibiotic Resistance of Pseudomonas aeruginosa*

Group 32

Aliasger Saifi, Shaheer Hassan, Eric Kang, Farhana Masood, and Sofia Czerny

Design Project TA: Mahnaz Tajik

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ABSTRACT

Antibiotics have played a huge role in the advancement of medicine to combat bacterial infections. However, with the misuse of these drugs, bacteria have developed resistance, rendering them ineffective. A synthetic biology system is proposed targeting one such bacteria, *Pseudomonas aeruginosa*. Various mechanisms are engineered into an *Escherichia coli K12* cell in order to track the bacteria, produce a toxic protein to kill the bacteria (Pyocin S5), and create a pathway to administer this protein. Using the SimBiology add-on in MATLAB, the proposed biological system is modeled and further simulated to test the efficiency of the engineered mechanism. Upon running the simulation for roughly 11 hours with an autoinducer ($3OC_{12}$-HSL) concentration of 1.0E-4 M, it is found that the greatest produced concentration of Lysis E7 and Pyocin S5 is 1.99E-18 M; insufficient for *P. aeruginosa* cell apoptosis.

Introduction

The introduction of antibiotics in the field of medicine has revolutionized the treatment of infectious diseases, and greatly increased the life expectancy of patients over the last century. Antibiotics repress the growth of harmful bacteria in the body or kill the bacteria itself through specialized targeting mechanisms [1]. However, with the increased and improper usage of these antimicrobial substances, bacteria have begun to develop ~~resistance to these drugs, known as~~ antibiotic resistance. One such example of a highly resistive bacteria is *Pseudomonas aeruginosa*, a gram-negative pathogen that causes severe chronic and acute infections throughout the body.

P. aeruginosa is a bacterium frequently linked to ventilator-associated pneumonia (VAP) in intensive care units (ICU) [2] and has shown to increase VAP mortality to 41.9% [3]. Eradication of the bacteria has become progressively difficult as it has multiple biochemical pathways working in conjunction to counter antibiotics. The bacteria has ~~been~~ shown to possess a high level of resistance to antibiotics through restricted outer membrane permeability, efflux systems that pump antibiotics out of the cell, and production of antibiotic-inactivating enzymes that render these medications useless against this deadly bacteria [4]. Due to the inability to rapidly and continuously develop revised antibiotics, new approaches are required ~~in order~~ to combat such challenges.

One ~~such~~ emerging field with great potential to combat *P. aeruginosa* is synthetic biology. Synthetic biology combines the fields of engineering and biology to redesign and enhance existing biological components with new abilities to accomplish unimaginable tasks [5]. Figure 1 outlines the proposed solution to target and eliminate *P. aeruginosa* ~~cells~~ using synthetic biology. In order to tackle this issue, a biological system was designed consisting of an engineered *Escherichia coli K12* cell with a built-in quorum sensing mechanism to detect *P. aeruginosa* biofilms at the site of infection. Through multiple mechanisms occurring within the engineered cell, the proposed system produces a toxic protein within the *E. coli K12* cell, ~~known as Pyocin S5~~. For this toxic protein to be released from ~~the~~ *E. coli K12* ~~cell~~, an exit mechanism is required. Alongside Pyocin S5, ~~another protein is produced which creates~~ pores in the cell membrane of ~~the~~ *E. coli K12* ~~cell, known as Lysis E7~~. Together, these proteins work to eliminate *P. aeruginosa* ~~cells~~ within the body. In order to monitor these processes, two downstream indicator proteins are used to signal the lysis of the cell membrane~~,~~ and production of the toxic protein [6]. Figure 3 ~~in the results and discussion section~~ highlights these mechanisms in greater detail.

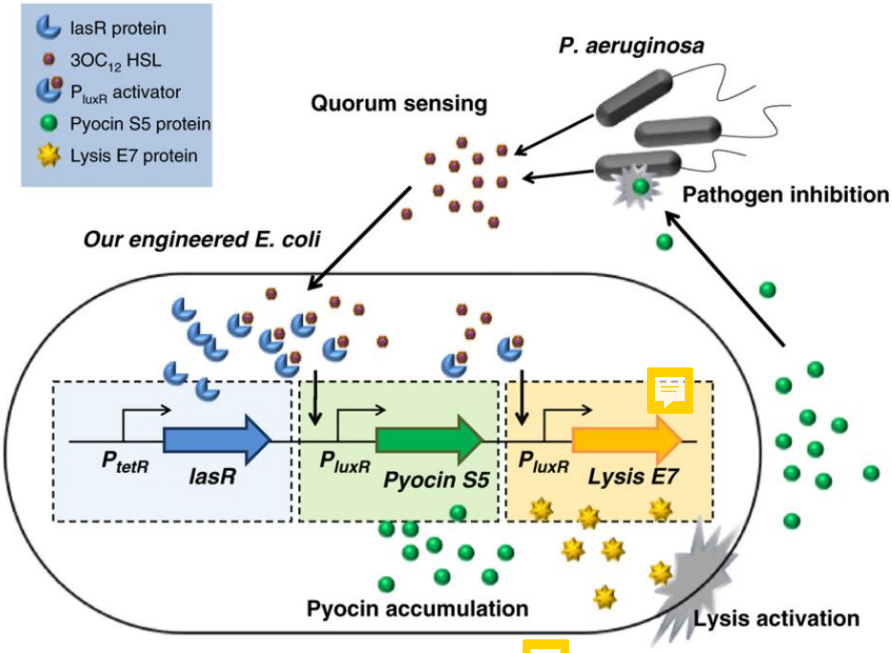

Figure 1. General schematic of proposed solution [7]

Materials and Methods

The biological parts required for the assembly and where they are retrieved from are included in Table 1.

Table 1. Biological parts required for model assembly

Biological Part	Source
Escherichia coli K12	iGem Parts Registry [8]
TetR Repressible Promoter	iGem Parts Registry [9]
LasR Gene	iGem Parts Registry [10]
Pet28b (plasmid backbone)	Millipore Sigma [11]
$3OC_{12}$-HSL (autoinducer)	iGem Parts Registry [12]
PLuxR (promoter for LasR-$3OC_{12}$-HSL binding)	iGem Parts Registry [13]
Pyocin S5 gene	iGem Parts Registry [14]
Green Fluorescent Protein gene	iGem Parts Registry [15]
E7 Lysis gene	iGem Parts Registry [16]
Red Fluorescent Protein gene	iGem Parts Registry [17]

SimBiology Model

To construct the biological system, the SimBiology add-on in MATLAB R2019b is used, as shown in Figure 2. Since LasR is constitutively expressed using the TetR promoter, the transcription rate for LasR mRNA is assumed to be constant. Additionally, the transcription rates for mRNA_P5/GFP and mRNA_LE7/RFP are assumed to be the same. The translation rates for LasR, P5, GFP, LE7, and RFP are also the same. The same assumption is made for the degradation rates of the aforementioned species. Additionally, mass-action kinetics for the binding/unbinding reaction between the $3OC_{12}$-HSL autoinducer and the LasR transcriptional activator is assumed. Finally, for reactions P5/GFP Transcription and LE7/RFP Transcription, Hill-Kinetics is assumed.

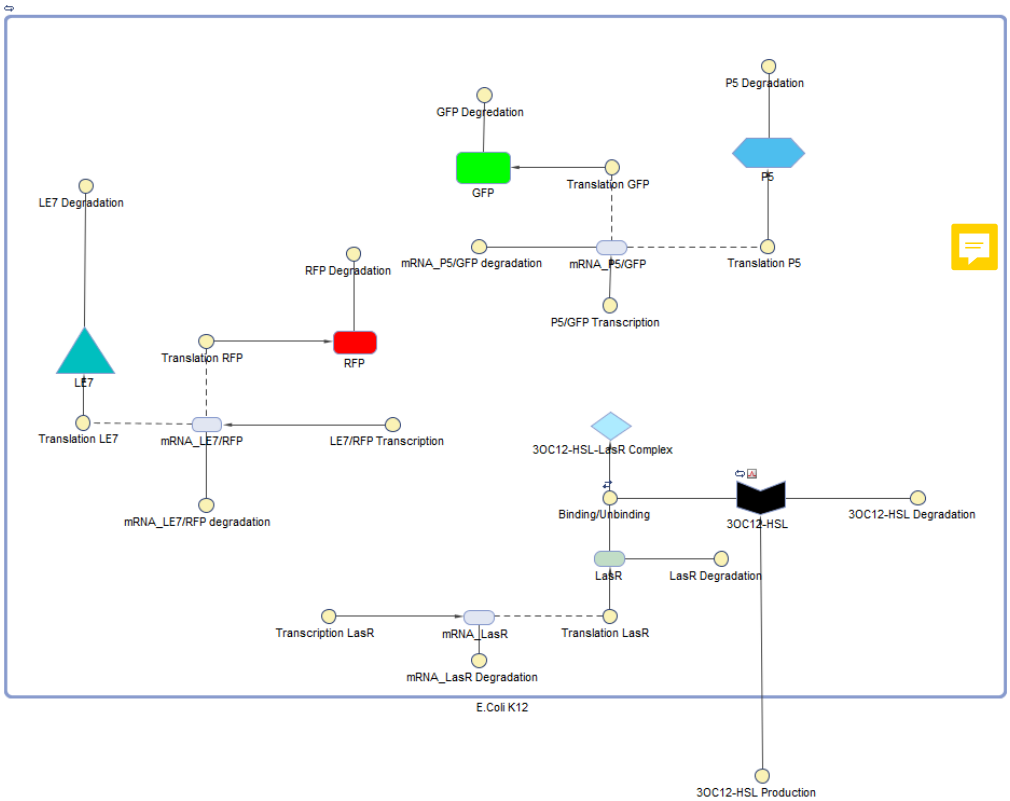

Figure 2. SimBiology schematic of processes occurring inside E. coli K12 cell

Wet-lab Experiments

The experiments conducted investigate whether the genetically engineered *E. coli K12* can locate and kill *P. aeruginosa* biofilms. To test the efficiency of the lysis device, the engineered plasmid is tagged with hexa-histidine on the 3' terminus of the S5 gene and is inserted into *E. coli K12* [6]. Overnight, cultures are harvested and then induced with 1.0E-6 M $3OC_{12}$-HSL and are incubated [6]. Once the produced proteins have been purified, the modified *E. coli K12* and *P. aeruginosa* are added in a co-culture agar plate at a 4:1, *E. coli* to *P. aeruginosa* ratio [6]. To test efficiency, fluorescent microscopy is used to calculate the percentage of cell survival [6]. The changes in bacterial culture can be determined using a spectrophotometer by measuring absorbance [6]. Furthermore, the elimination ability is dependent on the $3OC_{12}$-HSL dose [6]. Hence this experiment would be repeated with additional doses of 1.0E-5 M and 1.0E-4 M of $3OC_{12}$-HSL to test efficiency. Finally, percentage survival of *P. aeruginosa* is determined (Appendix Equation 1). The same procedure is repeated with two negative controls. The first being a modified *E. coli* that contains the Pyocin S5 gene while lacking the Lysis E7 gene. The second being a modified *E. coli* containing the Lysis E7 gene while lacking the Pyocin S5 gene. This should result in no changes to the *P. aeruginosa* biofilm as the system requires both, Pyocin S5 and Lysis E7 to be functional.

Results and Discussion

Figure 3 is a Synthetic Biology Open Language (SBOL) schematic demonstrating the biological system designed to counter the antibiotic resistance of *P. aeruginosa*. The host of this circuit is an *E. coli K12* cell, which was chosen due to its compatibility within the body, specifically the trachea, where most *P. aeruginosa* related infections occur. The system contains a quorum sensing mechanism that detects *P. aeruginosa* biofilms at the site of infection, causing the release of Pyocin S5 to kill the bacteria. This is done by recognizing the autoinducer produced by *P. aeruginosa*, $3OC_{12}$-HSL. Once the autoinducer diffuses into the cell, it meets LasR, a transcriptional activator, that is produced by the ~~constitutively on~~ TetR promoter. LasR ~~proceeds to~~ bind with the autoinducer, creating the LasR-$3OC_{12}$-HSL activated complex. The activated complex promotes the transcription of Pyocin S5 and Lysis E7, as well as their indicator proteins, GFP and RFP respectively, by binding onto the LuxR promoters. At high enough concentrations, Lysis E7 lyses the cell membrane, releasing Pyocin S5 which induces cell apoptosis in *P. aeruginosa* [6].

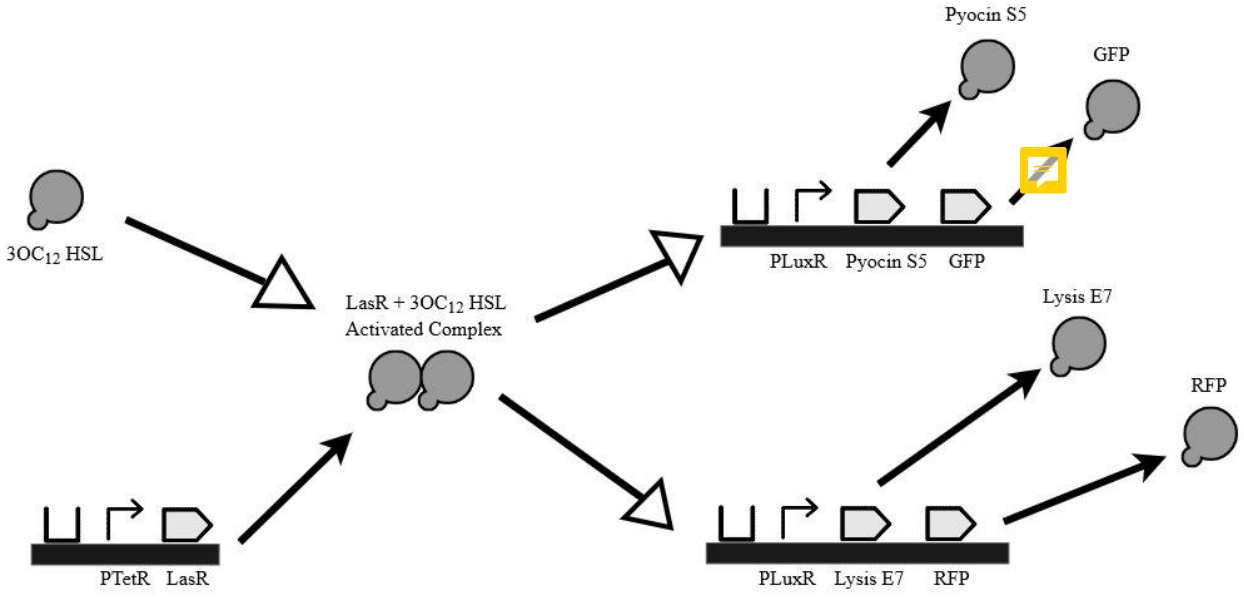

Figure 3. SBOL schematic of LasR-$3OC_{12}$-HSL activated complex producing Pyocin S5, Lysis E7, GFP and RFP in E. coli K12 cell

Figure 4 ~~is a graphical depiction of~~ the concentration of autoinducer, $3OC_{12}$-HSL, as a function of time. ~~As the graph depicts,~~ the simulation was run three times with differing initial concentrations of the autoinducer at 1.0E-6 M, 1.0E-5 M, and 1.0E-4 M. These concentrations were chosen as research indicates that the concentration of $3OC_{12}$-HSL at the site of *P. aeruginosa* infection ranges between 1.0E-6 M and 1.0E-4 M, ~~thus making them optimal operating concentrations for the sensing device~~ [6]. Additionally, as time increases, the concentration of autoinducer decreases exponentially, with the 1.0E-4 M sample decreasing at the greatest rate. This is a result of the binding of the autoinducer with LasR. As can be seen in Figure 4 all three lines eventually plateau, achieving steady state, which implies that the concentration of the autoinducer and LasR associating is equal to the concentration of the complex dissociating. For the 1.0E-5 M and 1.0E-6 M sample (lower two lines respectively), steady state is reached at around 20,140 seconds, and the concentration of $3OC_{12}$-HSL at these points differ by an order of magnitude. Though the greater concentration, 1.0E-4 M, does not reach steady state in this runtime, it is more beneficial in the complexation reaction as there is more autoinducer available to bind with LasR, thus forming more complex and more transcriptional products.

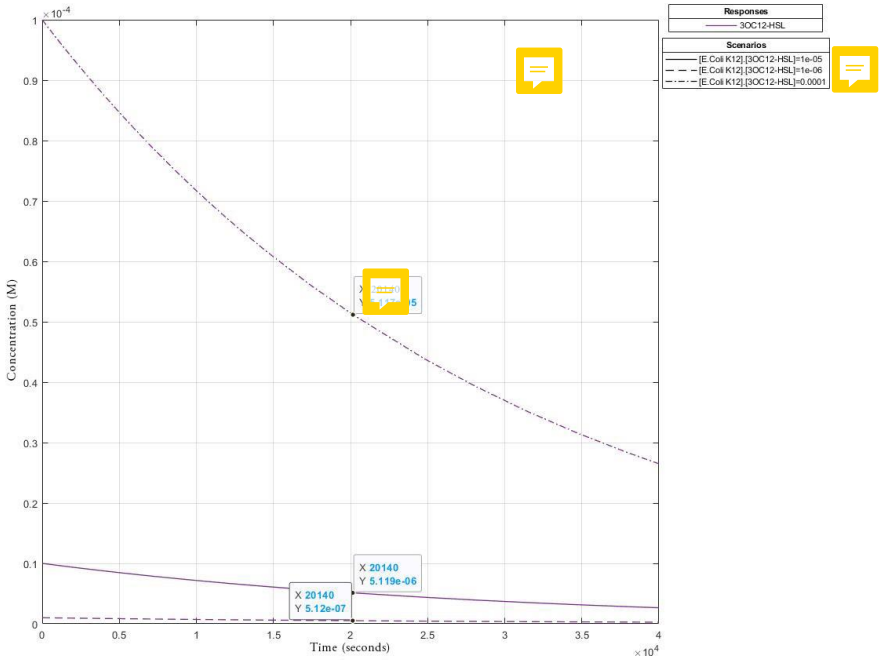

Figure 4. Graphical Representation of Concentrations 1.0E-6 M, 1.0E-5 M, and 1.0E04 M of $3OC_{12}$-HSL with Respect to Time (s)

Figure 5 ~~is a graphical depiction of~~ the concentration of LasR as a function of time. The graph clearly depicts that the concentration of LasR increases exponentially with time, beginning to reach steady state at approximately 4.0E4 seconds. Since LasR is continuously produced from an initial concentration of 0, with transcription and translation rates of 0.282M/s and 2.7778E-6 s^{-1} respectively, steady state is reached when the rate of production is equal to its rates of degradation and complex formation [18, 19]. This is because the SimBiology equations demonstrate a linear relationship between concentration and the rate of production and degradation. Furthermore, research studies have indicated that the time required for Lysis E7 to induce *E. coli* cell apoptosis is approximately 8,100 seconds. As indicated in Figure 5, the concentration of LasR at this point is 0.4367 M, further signifying that there is a sufficient concentration of LasR for the LasR-$3OC_{12}$-HSL binding reaction to proceed. However, a limitation with this amount of time is the doubling rate of *E. coli* cells, which ranges from 20 minutes in a lab to 24 hours in the wild [20]. Since the cell is expected to lyse after 135 minutes, performing an experiment with this circuit in a lab setting might be unrepresentative of the circuit's behaviour outside of lab conditions. However, the opportunity for in vivo and surface testing remains.

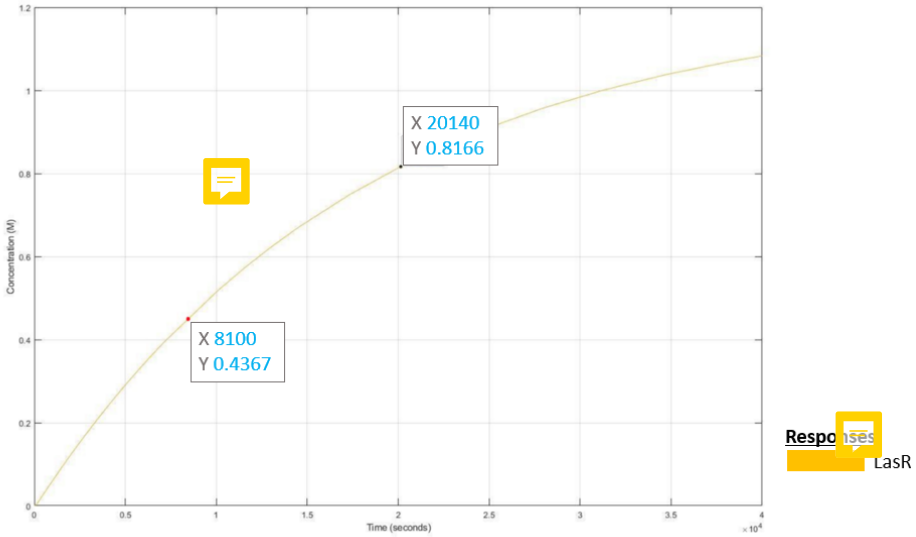

Figure 5. Graphical Representation of the Concentration (M) of LasR with Respect to Time (s)

Figure 6 displays the concentration of the LasR-3OC$_{12}$-HSL activated complex with respect to time. The three lines on the graph are indicative of the initial concentrations of the autoinducer. The complex forms with the forward binding rate of 3OC$_{12}$-HSL being 1.3E-6 M [18] and reverse binding rate being 7.69E-13 M (Appendix Equation 3). For all three concentrations, the maximum concentration of the complex is reached at approximately 20,140 seconds. When the concentrations of the complex reach a maximum, it also implies that the amount of autoinducer and LasR available for association is maximized. This occurs at 20,140 seconds, with a LasR concentration of 0.8166 M and the greatest 3OC$_{12}$-HSL concentration being 5.117E-5 M. The limiting factor is the concentration of 3OC$_{12}$-HSL, as Figure 4 shows a continuous exponential decay of 3OC$_{12}$-HSL concentration over time. Maximum attainable concentration of complex is limited by *P. aeruginosa'* s production of autoinducer as the circuit is dependent on its presence in order to function. It is also important to note that the rate of increase for complex concentration differs depending on the initial 3OC$_{12}$-HSL concentration. The 1.0E-4 M concentration allowed a much greater rate of increase compared to the 1.0E-5 M and 1.0E-6 M concentrations. This is significant because it determines the rate at which Pyocin S5 and Lysis E7 are produced.

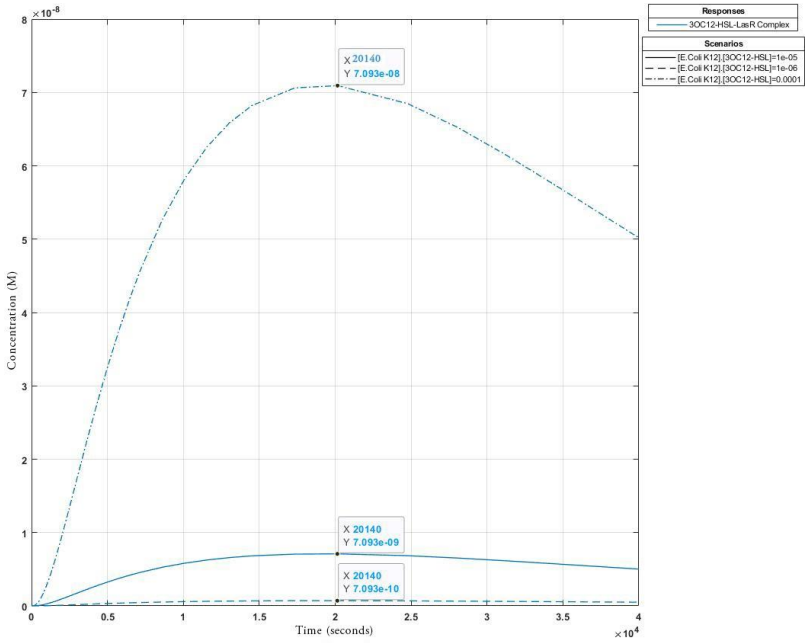

Figure 6. Graphical Representation of the Change in Concentration (M) of LasR-3OC$_{12}$-HSL Complex with Respect to Time (s)

Figure 7 shows the concentration of Pyocin S5, GFP, Lysis E7 and RFP with respect to time. As explained previously, the maximum concentration of the complex is reached at 20,140 seconds. At the time, the concentration of these four molecules is increasing at the greatest rate. Since the four proteins are assumed to be produced at the same rate of 2.7778E-6 s^{-1}, they overlap one another in Figure 7. A higher initial concentration of $3OC_{12}$-HSL causes the concentration of Pyocin S5 and Lysis E7 to increase more quickly. This allows the desired concentration of Pyocin S5 to accumulate more rapidly, thus resulting in earlier cell apoptosis compared to using lower initial concentrations of $3OC_{12}$-HSL. The desired concentration of Pyocin S5 is 4.01E-5 M (Appendix Equation 4). However, even after 40,000 seconds, the concentration reached by the four proteins is significantly lower than required, with the highest concentration being 1.99E-18 M for the $3OC_{12}$-HSL tested with an initial concentration of 1.0E-4 M. At this point, the system reaches steady state as shown by the plateau in Figure 7. To allow Pyocin S5 to reach its desired concentration, a higher initial concentration of $3OC_{12}$-HSL is likely required. Since there is insufficient research describing the concentration of Lysis E7 necessary to cause cell apoptosis, the bacterial culture would need to be run under spectrophotometry to see whether the concentration of *E. coli K12* decreases. This would provide information about whether cell apoptosis is occurring before the desired concentration of Pyocin S5 is reached. If this is the case, it is likely that the circuit would be ineffective as there would be insufficient Pyocin S5 to kill the *P. aeruginosa*.

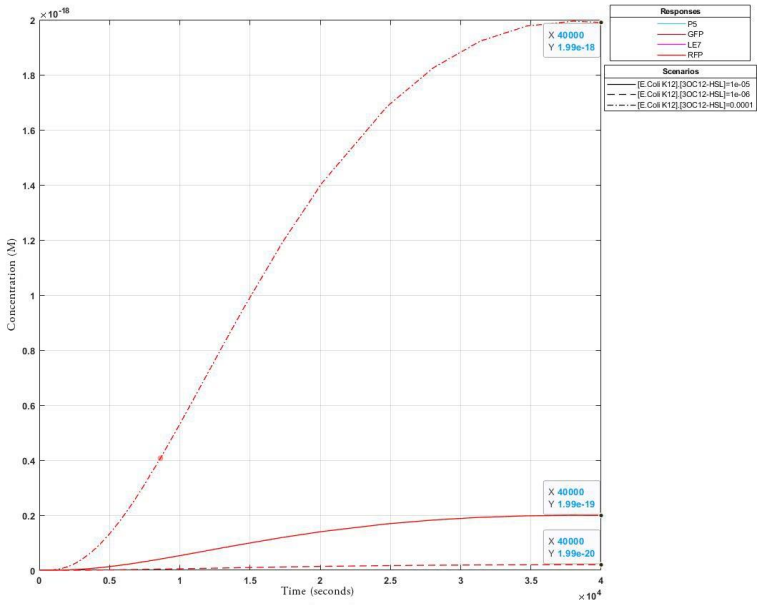

Figure 7. Graphical Representation of the Change in Concentration (M) of Species: P5, GFP, LE7, and RFP with Respect to Time (s)

Conclusion

In conclusion, it can be deduced that our engineered biological system may be unfeasible for eliminating *P. aeruginosa* biofilms. When the *E. coli K12* cells are induced with $3OC_{12}$-HSL, the system reaches maximum effectiveness when the induced concentration equals 1.0E-4 M. As a result of this, our system produces Pyocin S5 at a concentration of 1.99E-18 M. This proves unfeasible, as a concentration of 4.01E-5 M is required to effectively induce cell apoptosis in *P. aeruginosa*. Furthermore, at the end of the simulation, the Lysis E7 concentration is also 1.99E-18 M. This is a result of the assumption made that that Pyocin S5 and Lysis E7 have the same transcription, translation, and degradation rates. While it may simplify the SimBiology model, it limits the accuracy of the simulation. Furthermore, the simulations were run for 40,000 seconds. This was to ensure that all species being produced reached a steady state concentration. As indicated in Figures 4-7, all species reach steady state concentrations at their respective times. However, this time proves to be a limiting factor with respect to the wet-lab testing scenario.

To improve the model's accuracy, future SimBiology models will showcase the relationship between the engineered *E. coli K12* and *P. aeruginosa*. This will be done by accounting for the interaction between Pyocin S5 and *P. aeruginosa* with the goal of observing cell apoptosis. Furthermore, the system will use more accurate constants for each reaction (i.e. transcription, translation, degradation) as opposed to assuming the same rate constants as is done currently. Lastly, the health risks associated with fostering engineered *E. coli K12* in the human body provides an opportunity to explore different methods of delivering the intervention. Specifically, looking into the possibility of integrating the biological circuit with medical cleaning methods used to sanitize hospital equipment such as ventilators. With this, it may be possible to prevent *P. aeruginosa* from infecting a patient in the first place. Overall, synthetic biology provides new opportunities that antibiotics fail to, and our circuit is just one example of how to take advantage of that and become more resistant to resistance.

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Appendix

Table 2. Equations used in MATLAB for produced results

Reaction	Equation
Transcription LasR	k_{t1}
mRNA_LasR Degradation	$k_{d1} * mRNA_{LasR}$
Translation LasR	$k_{t1} * mRNA_{LasR}$
LasR Degradation	$k_{d2} * LasR$
Binding/Unbinding	$k_f * [3OC_{12} - HSL] * LasR - k_r \times [3OC_{12} - HSL - LasR\ Complex]$
$3OC_{12}$-HSL Degradation	$k_{d3} * [3OC_{12} - HSL]$
P5/GFP Transcription	$\frac{k_{trsc} * (\frac{[3OC_{12} - HSL - LasR\ Complex]}{K})^h}{1 + (\frac{[3OC_{12} - HSL - LasR\ Complex}{K})^h}$
mRNA_P5/GFP Degradation	$k_{d1} * [mRNA_{P5/GFP}]$
Translation GFP	$k_{t1} * [mRNA_{P5/GFP}]$
Translation P5	$k_{t1} * [mRNA_{P5/GFP}]$
GFP Degradation	$k_{d2} * GFP$
P5 Degradation	$k_{d2} * P5$
LE7/RFP Transcription	$\frac{k_{trsc} * (\frac{[3OC_{12} - HSL - LasR\ Complex]}{K})^h}{1 + (\frac{[3OC_{12} - HSL - LasR\ Complex}{K})^h}$
mRNA_LE7/RFP Degradation	$k_{d1} * [mRNA_{LE7/REF}]$
Translation LE7	$k_{t1} * [mRNA_{LE7/REF}]$
Translation RFP	$k_{t1} * [mRNA_{LE7/REF}]$
RFP Degradation	$k_{d2} * RFP$
LE7 Degradation	$k_{d2} * LE7$

Table 3. Data table of species and reactions

Reaction	Constant
Transcription Rate of LasR (kt1)	0.282 M/s [18]
LasR mRNA Degradation Rate (kd1)	0.0116 s^{-1} [21]
Translation Rate of LasR (ktl)	2.7778E-6 s^{-1} [22]
LasR Degradation Rate (kd2)	5.555E-5 s^{-1} [19]
Forward Binding Rate of LasR and $3OC_{12}$-HSL (kf)	1.3E3 nM [18]
Reverse Binding Rate of LasR and $3OC_{12}$-HSL (kr)	7.69E-4 nM^{-1} [23]
$3OC_{12}$-HSL Degradation Rate (kd3)	3.32E-5 s^{-1} [18]
Transcription Rate of mRNA Lysis E7 (ktrsc)	8.33333E-15 M/s [24, 25]
Transcription Rate of mRNA RFP (ktrsc)	8.33333E-15 M/s [24, 25]
Translation Rate of RFP (ktl)	2.7778E-6 s^{-1} [22]
Translation Rate of Lysis E7 (ktl)	2.7778E-6 s^{-1} [22]
Lysis E7 Degradation Rate (kd2)	5.555E-5 s^{-1} [19]
Transcription Rate of mRNA Pyocin S5 (ktrsc)	8.33333E-15 M/s [24, 25]
Transcription Rate of mRNA GFP (ktrsc)	8.33333E-15 M/s [24, 25]
Translation Rate of Pyocin S5 (ktl)	2.7778E-6 s^{-1} [22]

Translation Rate of GFP (ktl)	2.7778E-6 s^{-1} [22]
GFP Degradation Rate (kd2)	5.555E-5 s^{-1} [19]
Pyocin S5 Degradation Rate (kd2)	5.555E-5 s^{-1} [19]
RFP Degradation Rate (kd2)	5.555E-5 s^{-1} [19]
mRNA_PS5/GFP Degradation Rate (kd1)	0.0116 s^{-1} [21]
mRNA_LE7/RFP Degradation Rate (kd1)	0.0116 s^{-1} [21]
LasR to PLuxR Affinity (K)	9.49E-4 s^{-1} [18]

List of Equations

$$Percentage\ cell\ survival = \frac{CFU\ of\ P.aeruginosa\ in\ treated\ sample\ at\ time\ t}{CFU\ of\ P.aeruginosa\ treated\ with\ WT\ E.coli\ at\ time\ t} * 100$$

Equation 1. Percentage survival of *P. aeruginosa* [6]

$$\frac{M}{h} \times \frac{h}{M * s} = \frac{1}{s}$$

Equation 2. Conversion from M/h to 1/s

$$K_a = \frac{1}{K_d}$$

Equation 3. Relationship between association and dissociation constant [26]

$$0.225\frac{g}{L} = M \times (56{,}07)\frac{g}{mol}$$

$$\frac{(0.225\frac{g}{L})}{(56{,}07)\frac{g}{mol}} = M\frac{mol}{L}$$

$$M = 4.01 \times 10^{-5}\frac{mol}{L}$$

Equation 4. Pyocin S5 molarity calculation [27]