Quantification and Comparison of Quorum Sensing Response to Various Quinolone Molecules in *Pseudomonas aeruginosa* and *Burkholderia thailandensis*

By Evrim Arslan

A thesis submitted to the

Graduate School-Newark

Rutgers, The State University of New Jersey

in partial fulfillment of requirements

for the degree of

Master of Science

Graduate Program in Chemistry

written under the direction of

Dr. Darren B. Hansen

and approved by

_____

_____

_____

_____

Newark, New Jersey

October, 2012

ABSTRACT

Quantification and Comparison of Quorum Sensing Response to Various Quinolone Molecules in *Pseudomonas aeruginosa* and *Burkholderia thailandensis*

By Evrim Arslan

Thesis director:
Dr. Darren B. Hansen

Bacteria have a quorum sensing mechanism that helps them communicate to other bacteria. It helps optimize activities such as altering behavior in response to other bacteria, facilitating cell-to-cell communication, and defeating competitors. By studying different quorum sensing signaling molecules in *Pseudomonas aeruginosa* and *Burkholderia thailandensis*, we have concluded that bacteria exert synergistic and antagonistic relationship in response to their environment. Bioluminescence studies have proven that different quinolones possess different characteristics. The difference in quorum sensing response between the *Pseudomonas* and *Burkholderia* species is largely dictated by the methylation of the quinolone ring named 4-hydroxy-3-methyl-2-alkylquinolines (HMAQs) rather than unsaturation of the alkyl chain of *Burkholderia* species. Also, our results indicate that there is little cross-talk between the quinolone quorum sensing molecules of both *P. aeruginosa* and methylated *Burkholderia* species. Furthermore, limited response to *N*-oxide quinolones indicates an attenuation of the quinolone quorum sensing response.

ACKNOWLEDGEMENTS

I would like to thank my advisor, Dr. Darren B. Hansen, for his guidance, endless help and patience. I would like to thank my committee members Dr. Phillip Huskey, Dr. Roger Lalancette, and Dr. James Schlegel for their advice. Lastly, I wish to thank my family for their support and encouragement.

TABLE OF CONTENTS

LIST OF FIGURES AND GRAPHS

Introduction

All species communicate through factors such as pheromones, visual signals, olfactory senses, auditory senses, and quorum sensing. Communication is required for some vital behaviors such as mating, controlling population density, gene expression, food finding, predator-prey interactions and more vital factors. Bacteria use quorum sensing to communicate within a population. Quorum sensing (QS) is a cell-to-cell communication to coordinate the behavior of a group of bacteria. There are four criteria for a molecule to be considered a quorum sensing molecule. These are (i) quorum sensing signals must take place during growth, under certain physiological conditions, and/or in response to environmental changes; (ii) the signal generated must be recognized by a bacterial receptor; (iii) a concerted response is created and; (iv) the cellular response extends beyond physiological changes required to metabolize the cell-to-cell signal molecule.[1] If any of these criteria are not fulfilled, a molecule cannot be considered a 'quorum sensing' molecule. For instance, once toxic bacterial metabolites reach a critical concentration, it triggers a stress response. However, this is not considered communication because the bacterial cells are simply responding to toxic molecules.[2]

Quorum sensing allows bacteria to respond to bacterial cell density and regulates gene expression. It does this by releasing chemical signals called autoinducers as bacterial cell concentration increases. This allows bacteria to observe their population and respond to their population by coordinating their behavior. Also, these bacteria can engage in intra and interspecies communication mechanisms to integrate a complex genetic network.[3] Some of these interactions can worsen infections, by increasing bacteria virulence factors. According to Miller, both Gram-negative and Gram-positive

bacteria use quorum sensing to regulate symbiosis, virulence, competence, bioluminescence, conjugation, antibiotic production, motility, sporulation, and biofilm formation.[4]

The first bacteria discovered to use quorum sensing signaling to control bioluminescence was the Gram-negative bacteria *Vibrio fischeri* found in the organs of marine squid.[6] These bacteria live in symbiotic association with eukaryotic host cells that provide the bacterium with a nutrient rich environment in return for light.[4] Light production occurs when HSL, *N*- (3-oxohexanoyl)-homoserine lactone, reaches a certain threshold activating the LuxR controlled luciferase gene cluster. Each host uses this light for a specific purpose. For instance, *Euprymna scolopes* (a squid) and *V. fischeri* live in a mutual relationship in which the bacteria is provided with a nutrient rich environment and the squid 'counter-illuminates' itself using the light from the bacteria as a way to avoid casting a shadow beneath the seawater on bright nights. On the other hand, *Monocentris japonicus* (fish) uses this light to attract a mate.[4]

Gram-negative quorum sensing bacteria have LuxI and LuxR regulatory proteins that comprise the acylhomoserine lactone (AHL) quorum sensing system. LuxI is the quorum sensing synthase enzyme that produces HSL. Conversely, LuxR binds AHL and activates the *luxICDABE* operon. AHL can freely diffuse across the cell membrane and as a result the concentrations of AHLs inside and outside the cell become similar. However, once the bacterial cell population increases, the AHL and LuxR interact exposing the LuxR DNA binding domain. Then, LuxR binds to the *luxCDABE* promoter and the transcription is activated. This results in light emission in the symbiotic bacteria

V. fischeri found in luminescent marine organims.[5] Some extensively studied Gram-negative bacteria known to be using quorum sensing systems are *V. fischeri, P. aeruginosa, Agrobacterium tumefaciens*, and *Erwinia carotovora*.

In Gram-positive bacteria, secreted peptides are used as QS molecules. In general, the number of QS peptides increase as the cell population density increases. The peptide signal is transported out of the cell with ATP-binding cassette (ABC) transporter.[4] A histidine sensor kinase protein detects the increasing extracellular concentration of the peptide signal.[4] Then, the sensor kinase is phosphorylated on a conserved histidine residue and the phosphoryl group is transferred to a response regulator protein that is phosphorylated on a conserved aspartate residue.[4] Phosphorylation of the response regulator activates the transcription of quorum sensor controlled genes.[4] Some extensively studied Gram-positive bacteria known to use quorum sensing systems are *Streptococcus pneumonia*, *Bacillus subtilis*, and *Staphylococcus aureus.*

There are many bacteria that use more than one quorum sensing signal system to allow for the control of multiple subsets of genes. For instance, *P. aeruginosa* uses two AHL systems called LasIR and RhIIR. When the concentration of AHLs is high, LasR binds its specific AHL to activate the gene expression. *rhlR* is one of the genes activated by this complex and encodes a second receptor called RhlR which promotes expression of specific RhlR promoted genes. Therefore, genes controlled by the LasIR are expressed before those regulated by the RhIIR system. This is why *P. aeruginosa* temporarily regulates virulence stages in hosts. **Figure 1** illustrates the interactions between the AHL quorum sensing systems in *P. aeruginosa.*

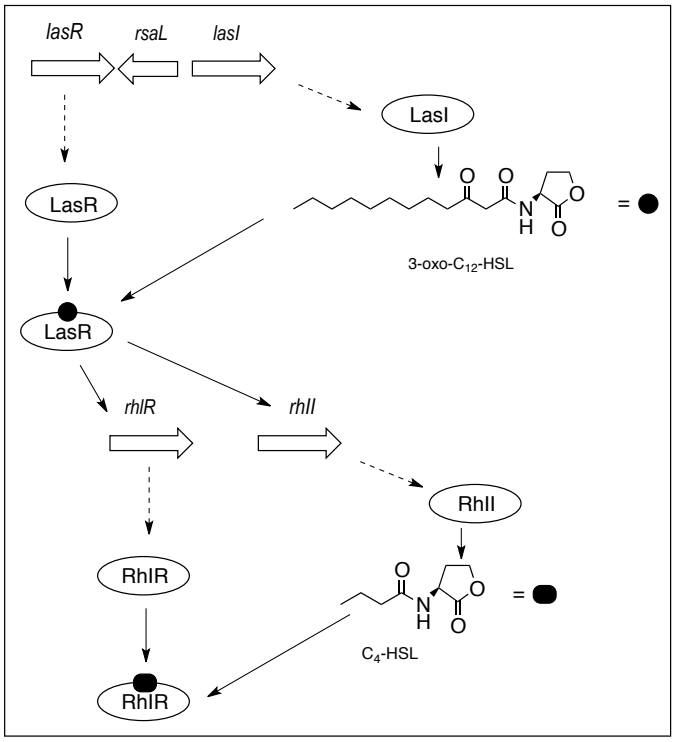
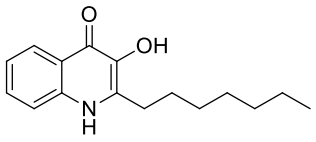


Figure 1. This figure illustrates the interaction between the AHL quorum sensing systems in *P. aeruginosa*.

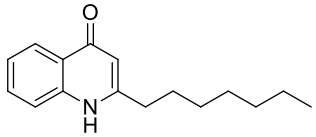
There is an additional QS molecule discovered in *P. aeruginosa* that is crucial in biofilm formation. This signal is *Pseudomonas* quinolone signal (PQS), 2-heptyl-3-hydroxy-4-quinolone, which is not part of the AHL system. PQS signal controls the expression of gene *lasB* along with Las and RhI quorum sensing systems.[4] It was discovered that PQS is an important secondary regulatory signal in genes controlled by quorum sensing that occurs temporally after AHL signaling cascade.



PQS

Figure 2. *Pseudomonas* quinolone signal (PQS)

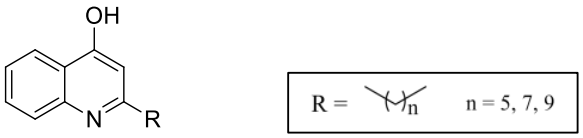
4-hydroxy-2-heptylquinoline (HHQ) is the direct precursor of PQS and is also involved in cell-to-cell communication.



HHQ

Figure 3. 4-hydroxy-2-heptylquinoline (HHQ)

HHQ and PQS can be found in the lung exudates of patients with cystic fibrosis.[7] This indicates that 4-hydroxy-2-alkylquinolines (HAQ) and PQS are produced *in vivo*. *P. aeroginosa* isolates from CF patients show more PQS production than isolates from other diseases suggesting that the PQS pathway is up regulated in CF patients.[8]

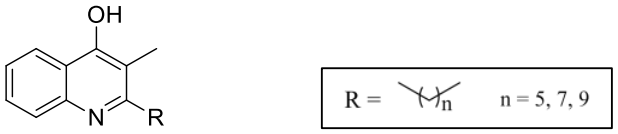


HAQ

Figure 4. 4-hydroxy-2-alkylquinolines (HAQ)

The protein MvfR has been identified as a virulence associated transcription factor that is involved in the production of PQS.[7] By using mass spectrometry to study HAQ congeners, it was discovered that they all have the basic 4-hydroxyquinoline structure with hydroxyl at the 3-position or an *N*-oxide group attached. The most common congeners include odd carbon number alkyl chains such as C7 and C9.[7] HAQ are not *N*-oxide precursors because adding 5,6,7,8-tetradeutero-4-hydroxy-2-heptylquinolone (HAQ-D_4) to *P. aeruginosa* strain does not result in production of tetradeutero *N*-oxides.

It has been documented that *Burkholderia cepacia* is also involved in severe respiratory infection in CF. *B. cepacia* is an opportunistic human pathogen that shares similar environments as *P. aeruginosa.* It is discovered that *B. cepacia* HAQs are methylated at position 3, hence their designation as 4-hydroxy-3-methyl-2-alkylquinolines, HMAQs.[9]



HMAQ

Figure 5. 4-hydroxy-3-methyl-2-alkylquinolines (HMAQ)

Both *P. aeruginosa* and *Burkholderia* produce quinolone quorum sensing (QQS) molecules involved in cell-to-cell communication. We hypothesize that methylation and nitrogen oxidation serve to modulate the quinolone quorum sensing response. Methylation of the 3' position by *B. thailandensis* serves to prevent cross-talk with *P. aeruginosa*. Nitrogen oxidation of the QQS molecules form N-oxides and serve as a method to attenuate or down regulate QS.

To study this hypothesis, we have used a combination of chemically synthesized QQS molecules and naturally extracted mixtures of QQS from both *B. thailandensis* and *P. aeruginosa* in conjunction with a genetically engineered *P. aeruginosa* reporter strain that emits light in response to QQS molecules allowing for easy quantification of QQS response to various quinolone molecules.

Materials and Methods

Stocks of the bacterial strains were purchased from the American Type Culture Collection (ATCC) and kept at -80 °C. Absorbance readings were measured at 600nm.

Luminescence was measured with a Tecan infinite 200 microtiter plate reader (Tecan Austria GmbH, Austria). All assays were performed in triplicate.

Bacterial cultures[10]

PA01, PA01 Δ*pqsA*, and PA01 Δ*pqsH* Quinolone Extraction

- First, each bacterium was streaked out onto Luria-Bertani (LB) agar plates and left to grow overnight at 37 °C.
- Next day, a single colony for each strain was inoculated into 50 mL of LB media in a 250 mL baffled flask and cultured overnight at 37 °C at 150 r.p.m. Optical density at 600 nm (OD_{600}) for each culture was determined and was diluted to OD 1.0 with fresh LB media. Standardizing the culture to OD_{600} 1.0 allows the comparison of different cultures for 2-alkyl-4-quinolones (AHQ) production.
- Then, 250 microliters of PA01, PA01 Δ*pqsA*, PA01 Δ*pqsH* cultures were transferred to 25 mL of LB medium in a 250 mL baffled flask and incubated overnight at 37 °C with shaking at 150 r.p.m. OD_{600} was recorded the next day.
- 15 mL of each culture was transferred to a 50 mL falcon tube and centrifuged at 2100x g for 15 minutes. Once the cells and the supernatants were separated, cell pellet extraction was done. To do this, 10 mL of methanol was added to the cell pellets allowing it to lyse. This was followed with centrifuging the samples at 2100x g for 15 minutes. The extracts were filtered through coffee filters and kept at -20 °C.
- 10 mL of acidified ethyl acetate was added to the supernatants and vortexed for 30 seconds to mix well. The mixtures were transferred to separatory funnels to allow for two phases to separate.

- The top organic layer was transferred to a 50 mL round-bottom flask. 10 mL of acidified ethyl acetate was added two more times and all the organic layers were collected in the 50 mL round-bottom flask. This was done for the PA01, PA01 Δ*pqsA*, and PA01 Δ*pqsH* cultures.
- The organic layers were rotary evaporated to dryness. 3.0 mL of methanol was added to the round-bottom flasks before transferring the liquid to a 10 mL clean vial.
- This last step was repeated two more times and all the liquid was collected in the clean vial. These extractions were kept at -20 °C for several months.

Burkholderia thailadensis E264 Quinolone Extraction

- Tryptic soy broth was inoculated with a single colony and cultured overnight at 150 r.p.m at 37 °C.
- The next day, 42.5 mL of methanol was added and the sample was centrifuged at 2100x g for 10 minutes.
- The supernatant was acidified to pH of 3.0 with 6.0 N HCl. 30.0 mL of acidified ethyl acetate was added to the supernatant and organic layers were washed with brine and collected in an Erlenmeyer flask.
- This last step was repeated two more times and the organic layers were all combined in the flask. The organic layers were rotary evaporated to dryness. 3.0 mL of methanol was added to the round-bottom flasks before transferring the liquid to a 10 mL clean vial.
- These extractions were kept at -20 °C for several months.

Compound preparation

Following compounds were used for the microplate QQS assay. Other members of the lab synthesized these compounds.

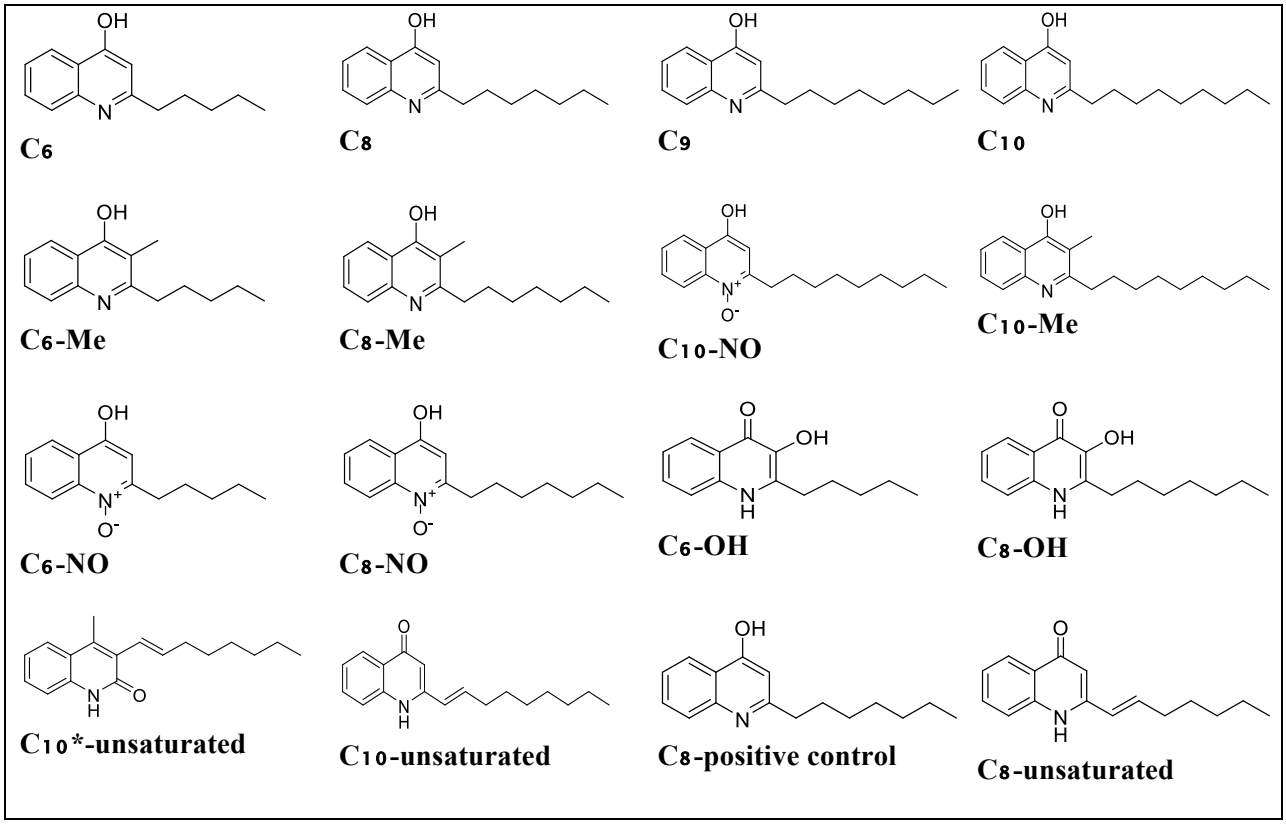


Figure 6. This figure lists the compounds used for the microplate QQS assay.

For each compound above, concentrations of 25µM, 5µM, 2.5µM, and 0.5µM from a stock of 10mM with MeOH were made. These were kept at room temperature.

Microplate QQS Bioassay

- 10 microliter of PA01 Δ*pqsA/lux* was streaked out on to a LB plate containing tetracycline and left to grow overnight.
- The next day, a single colony was inoculated and put into a 250 mL baffled flask containing 50 mL of LB medium containing tetracycline (30mg/mL). This was left to grow overnight at 37 °C with shaking at 150 r.p.m.

- The next day, this PA01 Δ*pqsA/lux* culture was diluted to OD (600 nm) 1.0 with fresh LB medium. The 1.0 OD_{600} was then diluted 1:100 with LB.
- Greiner 96 white flat bottom transparent polystyrol plate was sterilized under UV light for 15 minutes.
- In each well plate, 358 microliters of 1:100 dilution of bioassay culture-to-medium were added. For this plate, 358 microliters equals to 1 cm of path length.
- To each well plate that contained 358 microliters of bioassay-to-culture media, a 1 microliter bacterial extract or quinolone compound was added.
- The samples incubated at 37 °C with shaking at 15 minute intervals for 12 hours. Both the absorbance OD_{600} and luminescence were measured for each well.
- Luminescence was recorded as relative light units (RLU). The data were collected in Excel. Origin 8.1 (Northampton, Massachusetts) was used to graph the data.

Results

Bioluminescence is the emission of light by a living organism. It assists in quantifying and studying gene expression. The luminescence *lux* genes are contained in the *luxCDABE* operon. As seen in **Figure 7**, this operon is expressed when PQS binds to PqsR (MvfR) to enhance the PqsR binding to *pqsA* promoter.[10] *luxCDE* encodes a fatty acid reductase complex while *luxAB* catalyzes the luminescence reaction.[10, 12] Both Gram-negative and Gram-positive bacteria can be used as biosensors to emit luminescence in response to transcription of up regulation of this operon when inserted downstream of the desired promoter sequence.

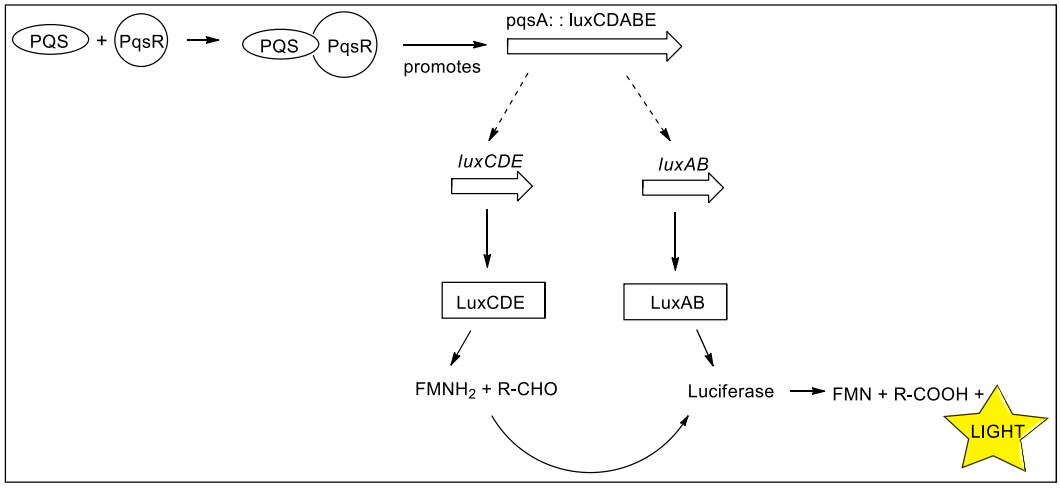
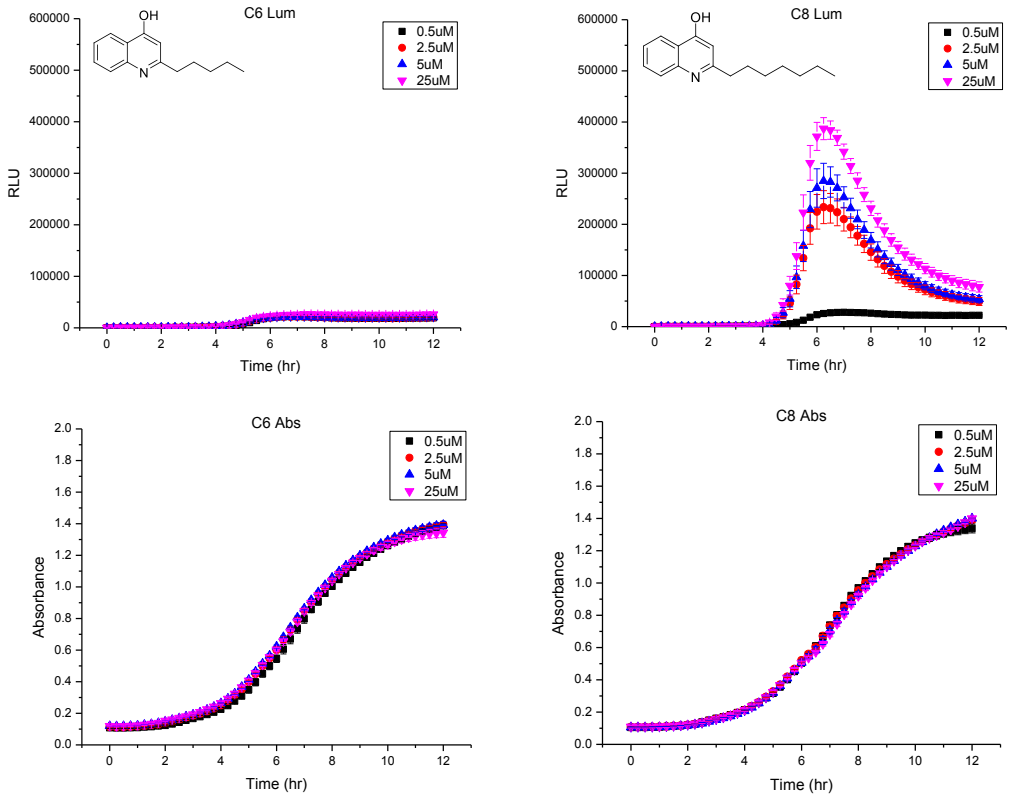


Figure 7. This figure illustrates the light production induced by PQS.

Hence, this method of investigating gene expression has been popular.[10,12] In the below luminescence graphs, it takes about 4 to 5 hours for start of logarithmic growth phase, at which point the concentration of bacteria grow rapidly. If the bioluminescence reporter is promoted, an increase in luminescence will be seen that correlates well with logarithmic increase in bacteria concentration. After 7 to 8 hours, the culture reaches a stationary phase and a decrease in luminescence is seen. Additionally, OD_{600} is recorded at regular time-points to ensure that all cultures are growing at the same rate and that no growth defects are observed during quinolone addition. In all assays performed, the absorbance curves reflect regular growth since the starting inoculum was standardized for all assays and no toxic side effects were observed. This allowed all the different cultures to be directly compared to one another. Overall, similar growth curves at OD_{600} are seen in each assay regardless of the compounds and bacterial extracts studied.

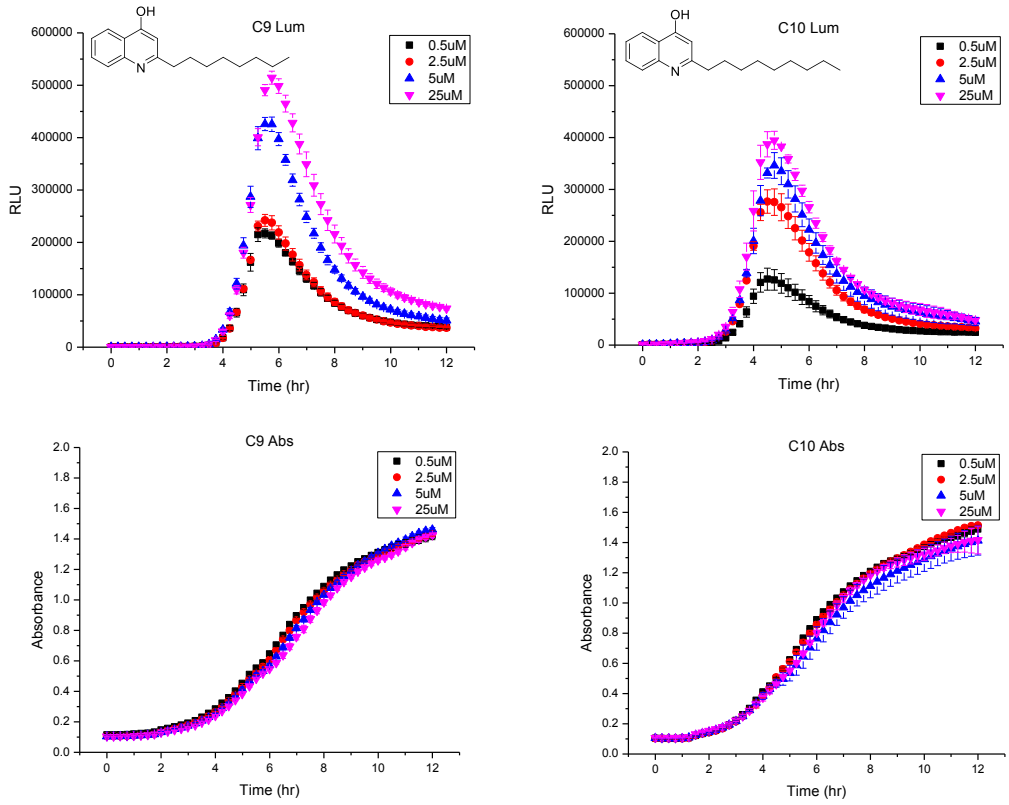
Luminescence and Absorbance for Saturated C₆ and C₈



Graph 1. Response of the QQS biosensor to saturated C₆ and C₈ alkyl quinolones. Top two graphs are dose-response curves showing luminescence from the QQS biosensor at concentrations of 25µM, 5µM, 2.5µM, and 0.5µM. Bottom two graphs are the absorbance curves for the same concentrations.

Response of the QQS biosensor to C₆ alkyl quinolone is not detectable above the background. This is because C₆ alkyl quinolones presumably do not bind pqsR and therefore do not upregulate the transcription of the *lux* operon. On the other hand, there is a high C₈ alkyl quinolone response from the QQS biosensor. As the concentration increases, so does the luminescence.

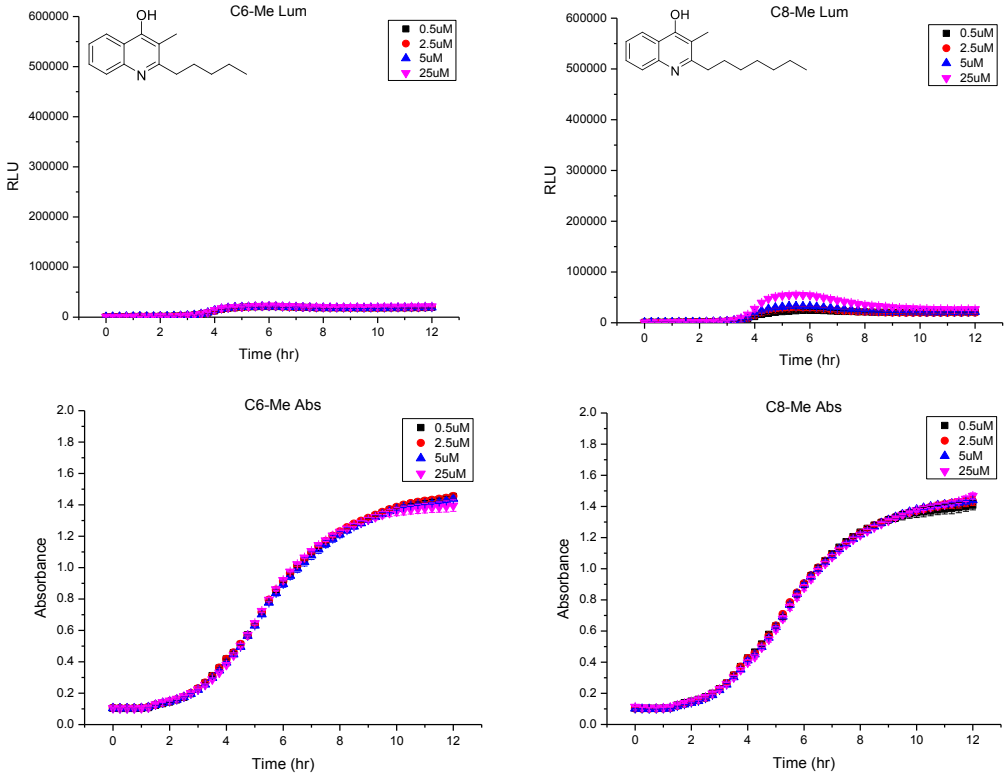
Luminescence and Absorbance for Saturated C₉ and C₁₀



Graph 2. Response of the QQS biosensor to saturated C₉ and C₁₀ alkyl quinolones. Top two graphs are dose-response curves showing luminescence from the QQS biosensor at concentrations of 25µM, 5µM, 2.5µM, and 0.5µM. Bottom two graphs are the absorbance curves for the same concentrations.

Both C₉ and C₁₀ alkyl quinolones produce high luminescence with C₉ showing a higher response for the concentrations of 25µM, 5µM, and 0.5µM than the C₁₀ alkyl quinolones. The paucity of C_9 quinolones in PA01 extracts is most likely related to limited number of odd number fatty acids produced in most bacteria. The strong response to C_9 quinolones shows that the QQS response and PqsR are sensitive to alkyl chain length but not specific to chain carbon numbers. Since C₈ and C₁₀ alkyl quinolones are most abundant in wild type *P. aeruginosa,* it is understandable to observe high response in C₁₀ alkyl quinolones.

Luminescence and Absorbance for Methylated C₆ and C₈



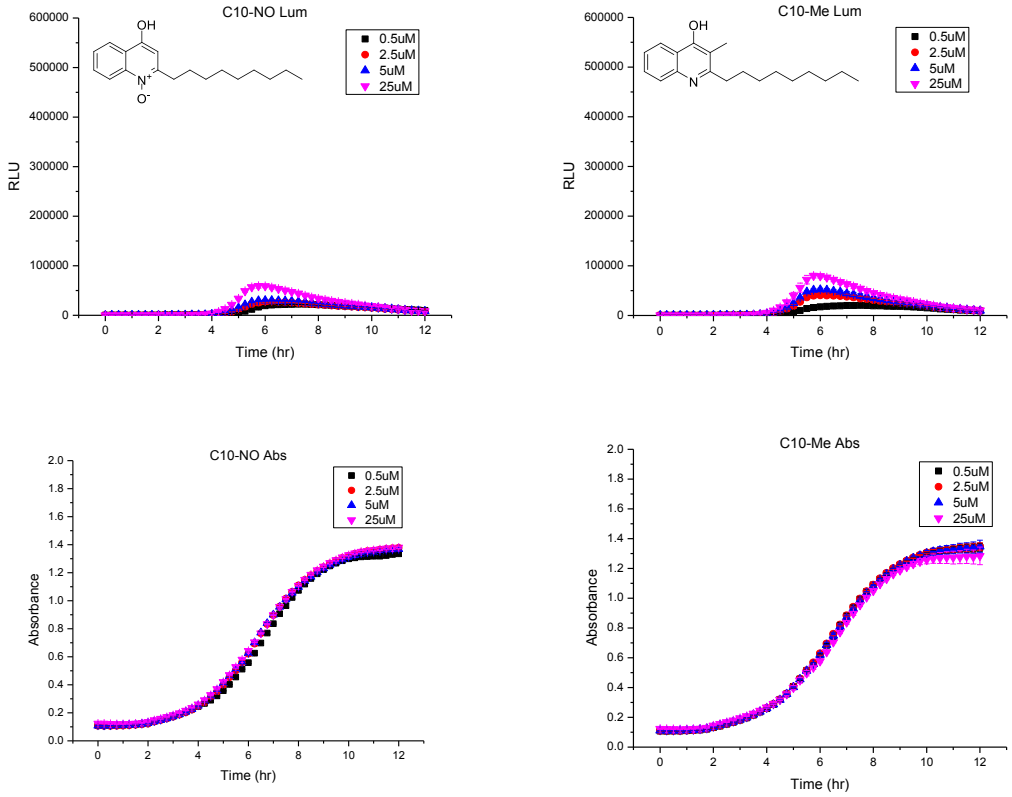
Graph 3. Response of the QQS biosensor to methylated C₆ and methylated C₈ alkyl quinolones. Top two graphs are dose-response curves showing luminescence from the QQS biosensor at concentrations of 25µM, 5µM, 2.5µM, and 0.5µM. Bottom two graphs are the absorbance curves for the same concentrations.

Burkholderia QQS molecules typically bear a methyl group hence are called 4-hydroxy-3-methyl-2-alkylquinolines (HMAQs). Methylation of the HAQ quinolone ring at the 3' position is hypothesized to be a result of a S-adenosyl-L-methionine (SAM) dependent methyltransferase encoded by the last gene of the *ABCDEFG* operon, *hmqG*.[9] This has a profound effect on the structure of AHLs produced in *Burkholderia* species.

Luminescence dose-response of QQS biosensor to methylated C₆ is nearly non-existent (**Graph 3**). For both methylated C₈ (**Graph 3**), and C₁₀ alkyl quinolones (**Graph 4**), minimal response is seen and is only 10-20% compared to the non-methylated analogues.

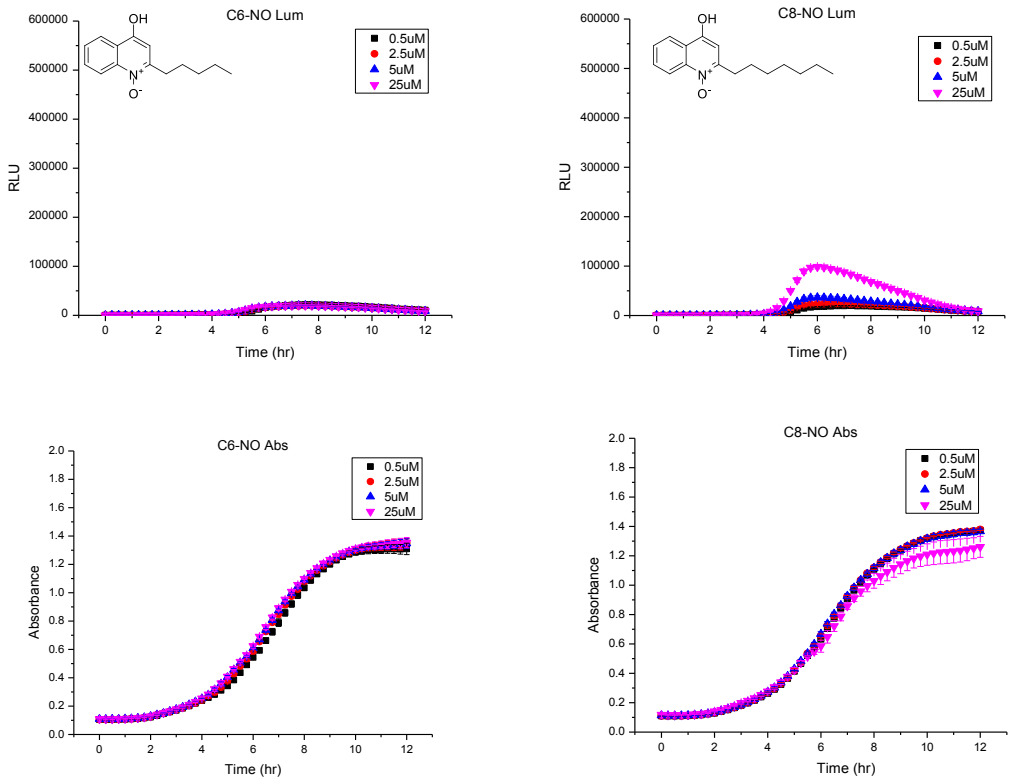
This is because 3'-methyl group effectively prevents cross talk between *Burkholderia* and *P. aeruginosa* species. This is presumed to be because the 3'-methyl group prevents or limits binding to PqsR and up regulation of bioluminescence.

Luminescence and Absorbance for C₁₀ N-oxide and C₁₀ Methylated



Graph 4. Response of the QQS biosensor to C₁₀ *N*-oxide and methylated C₁₀ alkyl quinolones. Top two graphs are dose-response curves showing luminescence from the QQS biosensor at concentrations of 25μM, 5μM, 2.5μM, and 0.5μM. Bottom two graphs are the absorbance curves for the same concentrations.

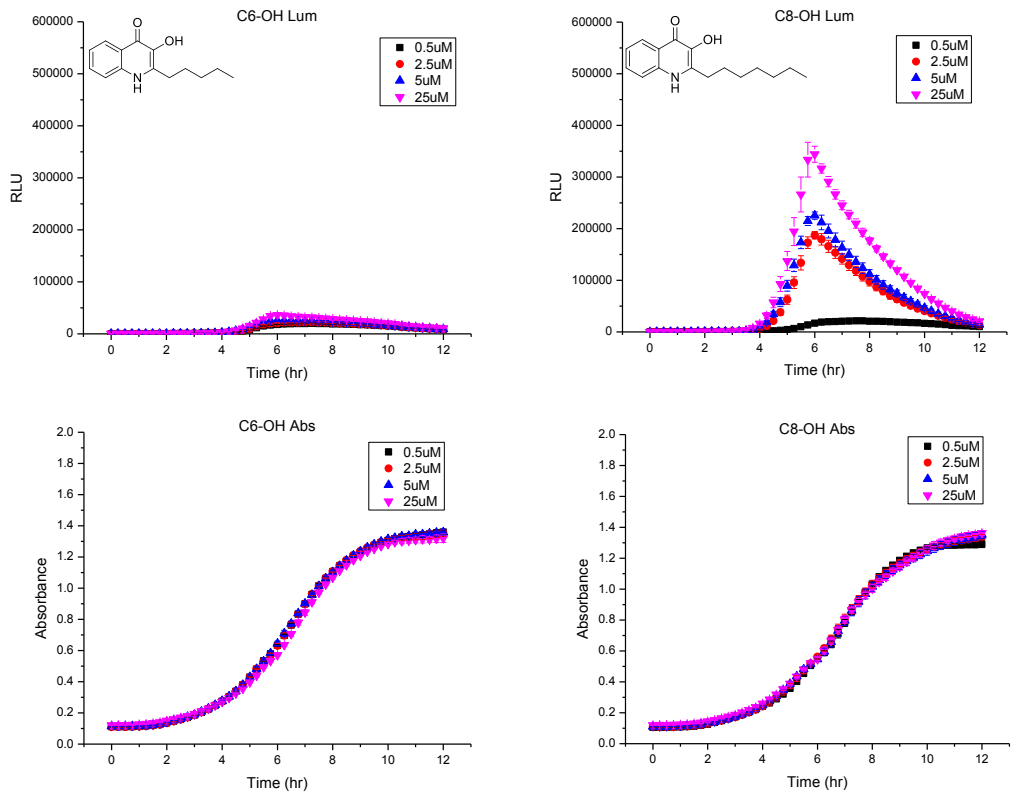
Luminescence and Absorbance for C₆ N-oxide and C₈ N-oxide



Graph 5. Response of the QQS biosensor to C₆ N-oxide and C₈ N-oxide alkyl quinolones. Top two graphs are dose-response curves showing luminescence from the QQS biosensor at concentrations of 25µM, 5µM, 2.5µM, and 0.5µM. Bottom two graphs are the absorbance curves for the same concentrations.

Quorum sensing response is attenuated in presence of *N*-oxide quinolones. *N*-oxide formation is hypothesized to be the first step in QQS degradation. As seen in **Graph 5**, QQS biosensor shows a limited response to C₆ *N*-oxide while it has a greater response to C₈ *N*-oxide alkyl quinolones. The response of the QQS drops for the C₁₀ *N*-oxide alkyl quinolone, **Graph 4**. This suggests *N*-oxide formation can attenuate QS response and may be a method to regulate QS. *N*-oxide formation is the result of the gene *pqsL* that encodes a FAD dependent monooxygenase.[11]

Luminescence and Absorbance for C₆ and C₈ Hydroxy

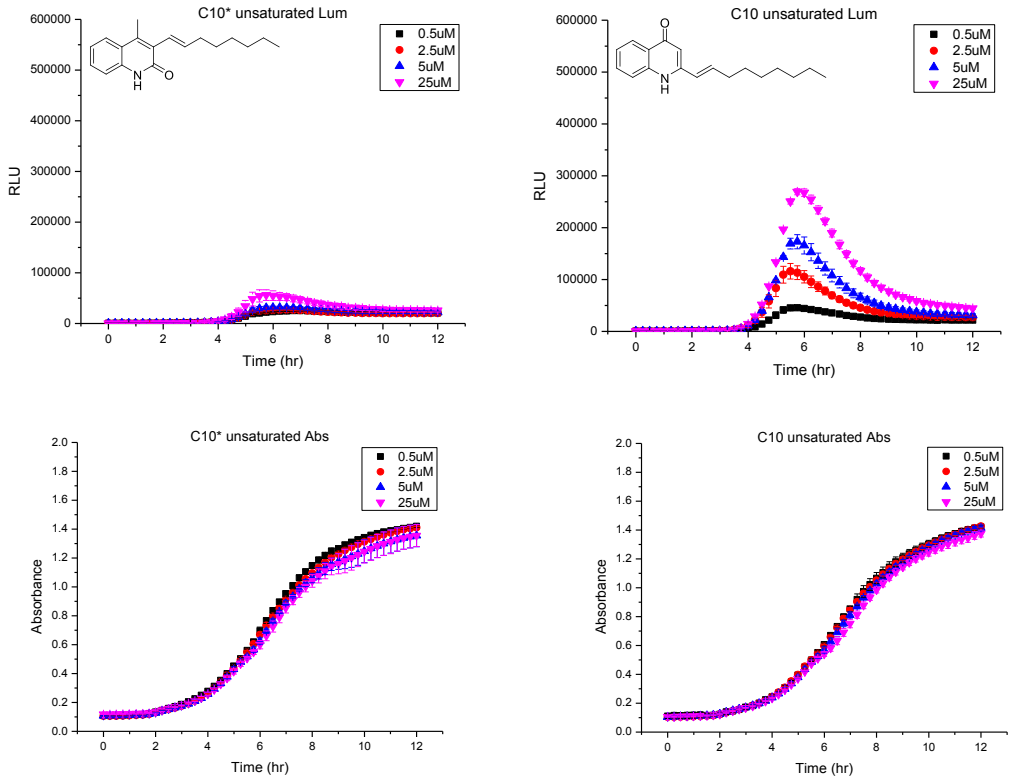


Graph 6. Response of the QQS biosensor to C₆-OH and C₈-OH alkyl quinolones. Top two graphs are dose-response curves showing luminescence from the QQS biosensor at concentrations of 25µM, 5µM, 2.5µM, and 0.5µM. Bottom two graphs are the absorbance curves for the same concentrations.

Hydroxylation of alkyl quinolones is chemically similar to conversion of HHQ to PQS. In **Graph 6,** hydroxylation of C₈ alkyl chain shows a higher luminescence than C₆ alkyl chain. This is mediated by the gene pqsH, a FAD monoxygenase. It is the gene pqsH and HHQ availability that both determine the PQS production. HHQs are produced by the lasR cells, which are taken up by the MvfR cells, and then converted into PQS.[7] For hydroxylated C₈ alkyl quinolones, 88% response is seen in comparison to the non-

hydroxylated analogue. On the other hand, for hydroxylated C_6 alkyl quinolones, an increase of 28% response is seen when compared to the non-hydroxylated analogue.

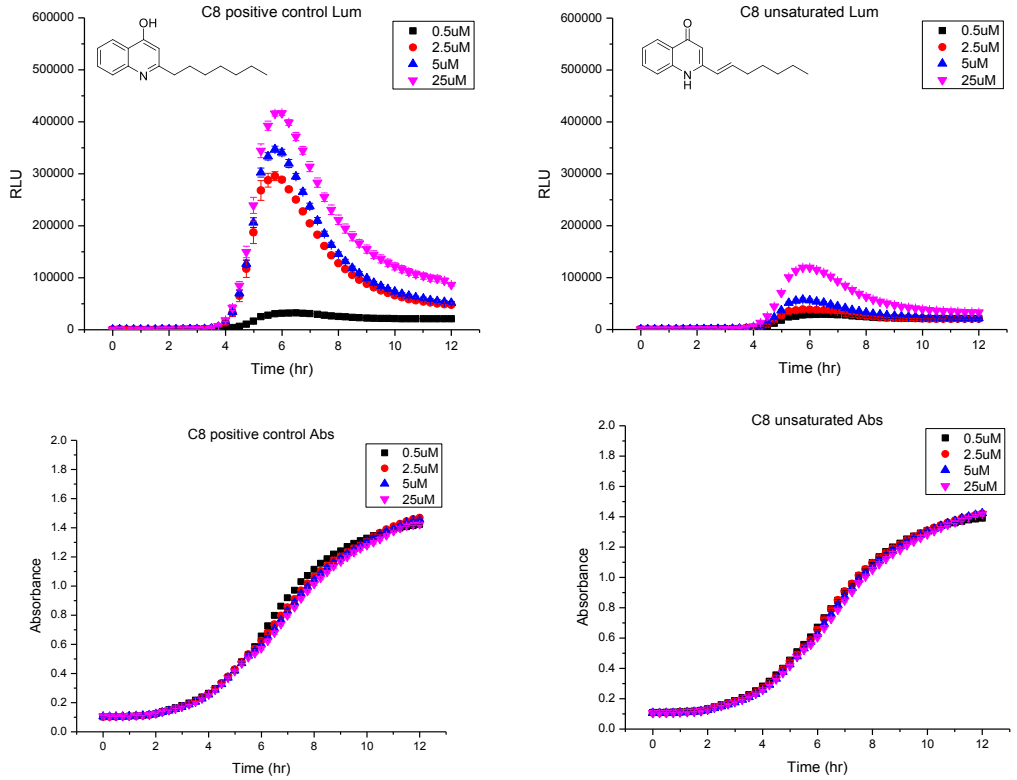
Luminescence and Absorbance for unsaturated C_{10} cyclized differently than unsaturated regular C_{10}*



Graph 7. Response of the QQS biosensor to unsaturated C_{10} cyclized differently and regular unsaturated C_{10} alkyl quinolones. Top two graphs are dose-response curves showing luminescence from the QQS biosensor at concentrations of 25µM, 5µM, 2.5µM, and 0.5µM. Bottom two graphs are the absorbance curves for the same concentrations.

Unsaturated C_{10} alkyl quinolones show a higher luminescence response than the one that is cyclized differently. This makes sense especially because the unsaturated alkyl quinolone that is cyclized differently has structurally a different quinolone core structure.

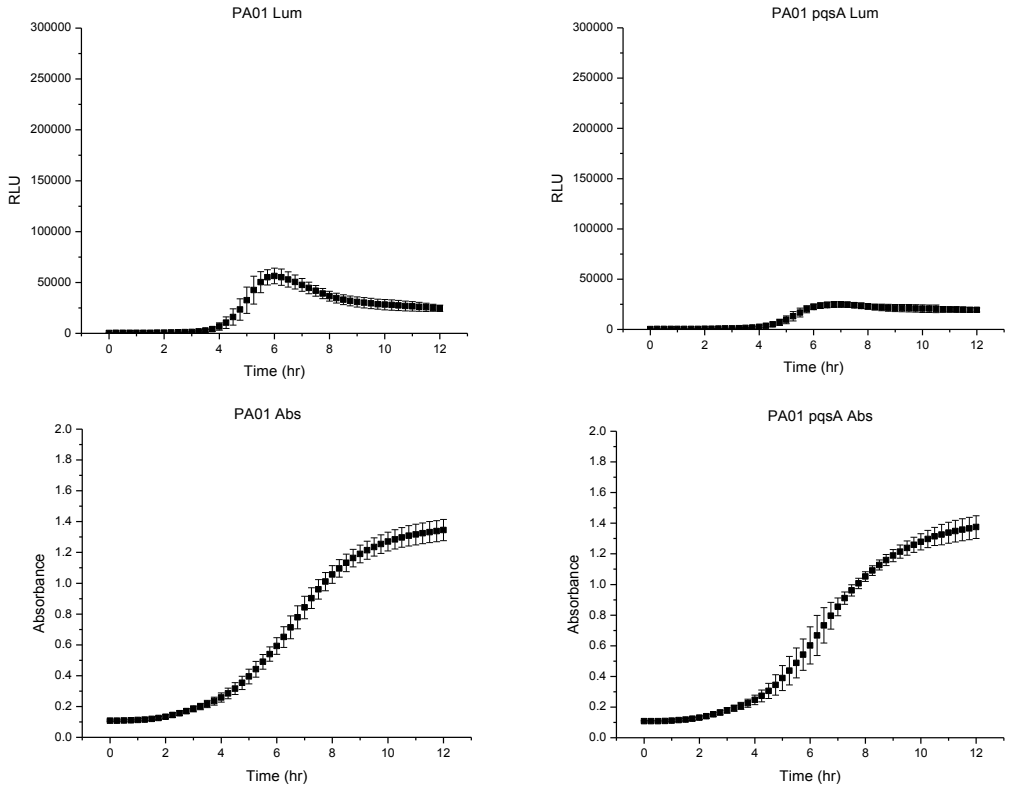
Luminescence and Absorbance for saturated C₈ (positive control) and unsaturated C₈



Graph 8. Response of the QQS biosensor to saturated C₈ (positive control) and unsaturated C₈ alkyl quinolones. Top two graphs are dose-response curves showing luminescence from the QQS biosensor at concentrations of 25µM, 5µM, 2.5µM, and 0.5µM. Bottom two graphs are the absorbance curves for the same concentrations.

Unsaturated C₈ alkyl quinolones show a lower luminescence than both the positive control and the unsaturated C₁₀ alkyl quinolones seen in **Graph 7**.

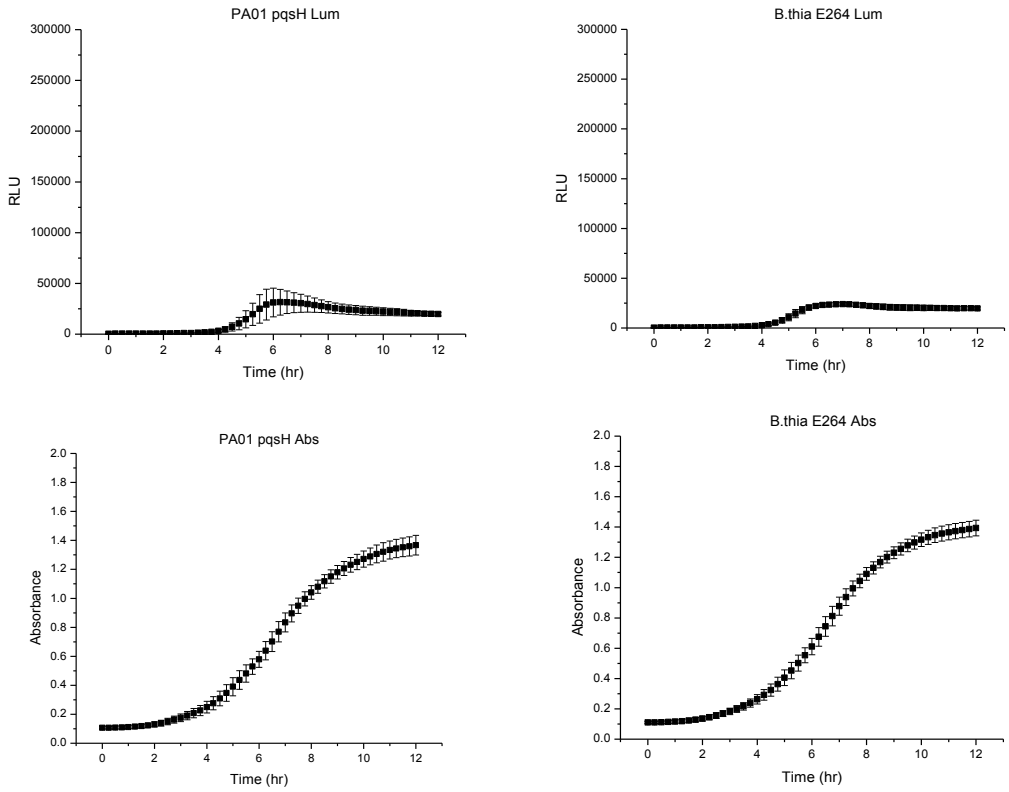
Luminescence and Absorbance for PA01 and PA01 ΔpqsA extracts



Graph 9. Response of the QQS biosensor to PA01 and PA01 Δ*pqsA*. Top two graphs are dose-response curves showing luminescence from the QQS biosensor and the bottom two graphs are the absorbance curves.

PA01 Δ*pqsA* is deficient for AHQ biosynthesis therefore supernatant extract will contain no QQS and serves as a negative control and no luminescence response is seen for the PA01 Δ*pqsA* extracts. Alternatively, PA01 is AHQ positive control. Therefore, a higher response is seen confirming the presence of AHQs in PA01 extracts.

Luminescence and Absorbance for PA01 *pqsH and B.thia E264 extracts*



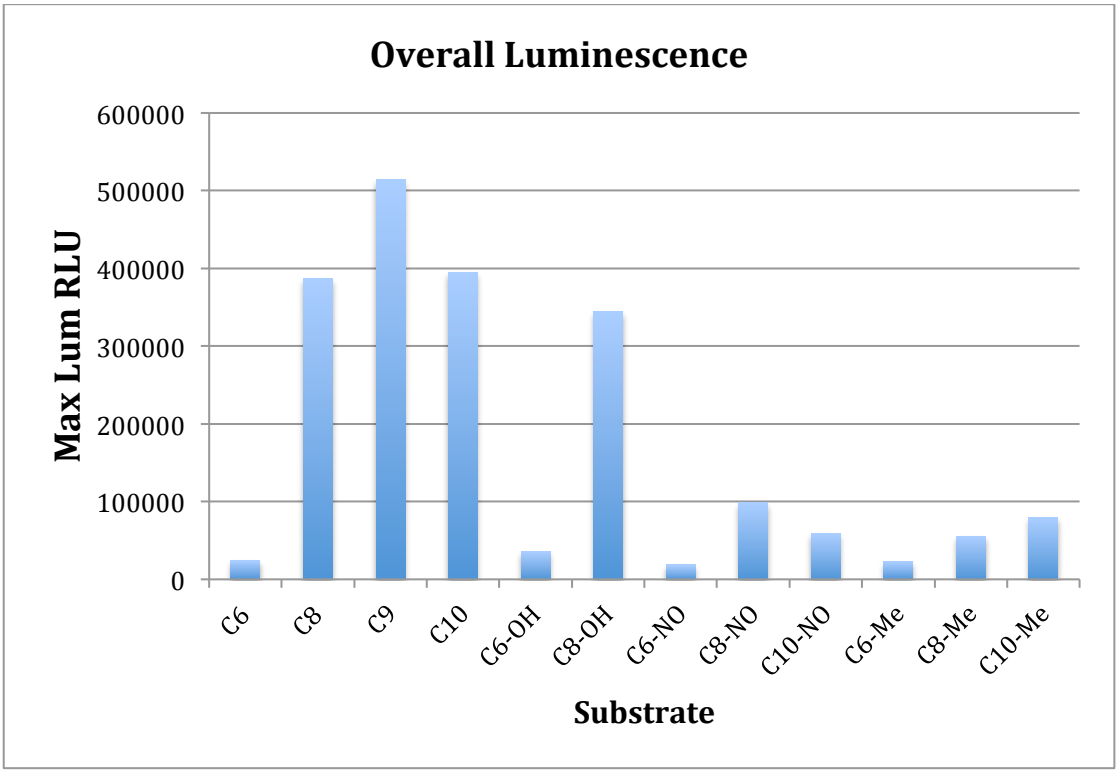
Graph 10. Response of the QQS biosensor to PA01 Δ*pqsH* and B. *thailandensis* E264. Top two graphs are dose-response curves showing luminescence from the QQS biosensor and the bottom two graphs are the absorbance curves.

PA01 Δ*pqsH* makes HHQ and related AHQs but not PQS or PQS analogues. It is HHQ positive control and PQS negative control. **Graph 10** has a very low dose-response to luminescence from the PA01 Δ*pqsH* extracts which is most likely related to lower amounts of quinolones in the extract since we were not able to standardize or quantify the amount of quinolones in extracts. The low response seen in this extract and wild type is most likely attributed to extremely low amount of quinolones present in the extract. In the future, the amount of quinolones need to be determined by another method to ensure standardized amount is used in each assay. The limited response in *B.thailandensis E264*

strain is because they produce methylated quinolone quorum sensing molecules almost exclusively.

Discussion

Our results indicate that there is little cross-talk between quinolones produced by *Burkholdeia* species and *P. aeruginosa*. The 3'-methyl group of *Burkholderia* species effectively prevents cross-talk. Furthermore, limited response to *N*-oxide quinolones supports the hypothesis that *N*-oxide formation is the initial step in quorum sensing attenuation.



Graph 11. Biosensor assay- dose responsive emission of light for different quinolone quorum sensing molecules

Response of the luminescence from the AHQ biosensor to saturated C_6, C_8, C_9 and C_{10} alkyl quinolones all differ since the response is chain length dependent but there

is a clear preference for chain lengths of eight carbons or longer. The *pqsA*::*luxCDABE* biosensor is most sensitively activated by HHQ and less sensitively activated by PQS analogues with alkyl chains varying from C1 to C11 and HHQ analogues of C11 alkyl chains.[14] According to our results, there is no response to C_6 alkyl quinolones while C_8, C_9 and C_{10} display a high response. Since C_8 and C_{10} alkyl quinolones are abundant in nature, their dose-response curve was expected. Further studies of C_{11} and longer chain alkyl quinolones may help us understand the effect of alkyl chain length on luminescence studies.

Furthermore, there is a limited response of the QQS biosensor to saturated *N*-oxide alkyl quinolones. In **Graphs 4** and **5**, C_8 *N*-oxide shows the highest luminescence while C_6 and C_{10} alkyl quinolones have limited response. For C_6 *N*-oxide alkyl quinolones, 75% response is seen in comparison to their HHQ analogue while for C_8 *N*-oxide it is 25% and for C_{10} *N*-oxide, it is 16%. Nevertheless, the response of *N*-oxide alkyl quinolones is not significant as compared to regular alkyl quinolones. According to Xiao et al, a putative mono-oxygenase *pqsL* is believed to be responsible for *N*-oxide formation.[13] Further analysis of *N*-oxide quinolones will help understand the effect of down regulating in quorum sensing.

B. thailandensis QQS molecules and their limited response is a result of the 3-methyl group and unsaturation. The methylation at the 3' position presumably prevents any *pqsH* analogue from hydroxylating these molecules and prevents PQS production in *Burkholderia* species or QQS cross-talk. Hence, in **Graph 10,** a limited response in QQS

biosensor to *B. thailandensis* is seen. As compared to *P. aeruginosa, Burkholderia* strains have a distinct genomic context of their operon, *hmqABCDEFG*. There is no homologue of the gene coding for the MvfR, and no putative LysR box is present nearby or within the promoter of the operon. [9] Therefore, it is clear that quorum sensing in *B. thailandensis* and *B. cepacia* are more complex because it involves multiple LuxIR homologues responsible for numerous signals involved in regulation. [9]

Overall, it is definite that response to QQS is specific and subtle changes in either the alkyl chain length or methylation of the HAQ quinolone ring both effect signaling. Further studies of *Pseudomonas* and *Burkholderia* species will unveil the details of the differences in their quinolone biosynthesis and the effect it has on quorum sensing.

References

1. Winzer, K.; Hardie, K. R.; Williams, P. "Bacterial cell-to-cell communication: sorry can't talk now- out to lunch!" *Current Opinion in Microbiology*. **5**, 216-222 (2002).

2. Williams, P.; Winzer, K.; Chan, W. C.; Camara, M. "Look who is talking: communication and quorum sensing in the bacterial world." *Philosophical Transactions of the Royal Society*. **362**, 1119-1134 (2007).

3. Dunn, A.K.; Handelsman, J. "Toward an understanding of microbial communities through analysis of communication networks." *Antonie Van Leeuwenhoek.* **81**, 565-574 (2002).

4. Miller, M.B.; Bassler, L.B. "Quorum sensing in bacteria." *Annual Reviews of Microbiology*. **55**, 165-199 (2001).

5. Taga, M.E.; Bassles, L.B. "Chemical communication among bacteria." *The National Academy of Sci.* **100**, 14549-14554 (2003).

6. Nealson, K. H.; Platt, T.; Hastings, J. "Cellular control of the synthesis and activity of the bacterial bioluminescent system." *Journal of Microbiology*. **104**, 313-322 (1970).

7. Deziel, E.; Lepine, F.; Milot, S.; He, J.; Mindrinos, M. N.; Tompkins, R. G.; Rahme, L. G. "Analysis of *Pseudomonas aerinosa* 4-hydroxy-2-alkylquinolines (HAQs) reveals a role for 4-hydroxy-2-heptylquinoline in cell-to-cell communication." *The National Academy of Sci.* **101**, 1339-1344 (2004).

8. Guina, T.; Purvine, S. O.; Yi, E. C.; Eng, J.; Goodlett, D. R.; Aebersold, R.; Miller, S. I. "Quantitative proteomic analysis indicates increased synthesis of a quinolone by *Pseudomonas aeruginosa* isolates from cystic fibrosis airways." *The National Academy of Sci.* **100**, 2771-2776 (2003).

9. Vial, L.; Lepine, F.; Milot, S.; Groleau, M. C.; Dekimpe, V.; Woods, D. E.; Deziel, E. "*Burkholderia pseudomallei*, *B. thailandensis*, and *B. ambifaria* Produce 4-Hydroxy-2-Alkylquinoline Analogues with a Methyl Group at the 3 position That Is Required for Quorum-Sensing Regulation." *Journal of Bacteriology*. **190**, 5339-5352 (2008).

10. Fletcher, M. P.; Diggle, S.P.; Camara, M.; Williams, P. "Biosenser-based assays for PQS, HHQ, and related 2-alkyl-4-quinolone quorum sensing signal molecules." *Nature Protocols*. **2**, 1254-1262 (2007).

11. D'Argenio, A.D.; Calfee, M. W.; Rainey, P. B.; Pesci, E. C. "Autolysis and Autoaggregation in *Pseudomonas aeruginosa* Colony Morphology Mutants." *American Society of Microbiology*. **184**, 6481-6489 (2002).

12. Winson, M.K.; Swift, S.; Hill, P. J.; Sims, C. M.; Griesmayr, G.; Bycroft, B. W.; Williams, P.; Stewart, G. "Engineering the *luxCDABE* genes from *Photorhabdus luminescens* to provide a bioluminescent reporter for constitutive and promoter probe plasmids and mini-Tn5 constructs." *FEMS Microbiology Letters.* **163**, 193-202 (1998).

13. Xiao, G.; Deziel, E.; He, J.; Lepine, F.; Lesic, B.; Castonguay, M.; Milot, S.; Tampakaki, A. P.; Stachel, S. E.; Rahme, L. G. "MvfR, a key *Pseudomonas aeruginosa* pathogenicity LTTR-class regulatory protein, has dual ligands." *Molecular Microbiology*. **62**, 1689-1699 (2006).

14. Fletcher, M.; Diggle, S. P.; Crusz, S. A.; Chhabra, S. R.; Camara, M.; Williams, P. "A dual biosensor for 2-alkyl-4quinolone quorum-sensing signal molecules." *Environmental Microbiology*. **9**, 2683-2693 (2007).

CURRICULUM VITAE

DATE AND PLACE OF BIRTH
August 30, 1984, Turkey

EDUCATION
Rutgers University, Newark, NJ, M.S., Chemistry, October 2012
Rutgers University, Newark, NJ, B.A., Biological Sciences, May 2007
Lodi High School, Lodi, NJ, June 2003

WORK EXPERIENCE
Passaic Valley Sewerage Commissions, Newark, NJ
Laboratory Technician, Aug. 2008-Present

- Perform Biochemical Oxygen Demand on in-plant and industrial samples
- Analyze Total Suspended and Volatile Suspended Solids
- Execute Chemical Oxygen Demand analysis
- Analyze harbor samples for *Enterococcus faecalis*, Fecal coliform, and *Escherichia coli*
- Prepare all necessary standards and execute analyses on quality control samples

PsychoGenics, Tarrytown, NY
Research Associate, Oct. 2007-Oct.2010

- Analyzed data and potential deviations
- Executed behavioral and pharmacological experiments with mice
- Prepared drugs according to SOP's
- Purchased new laboratory equipment and supplies

RESEARCH EXPERIENCE
Chemistry Department, Rutgers University, Newark, NJ
Independent Study, July 2010- Present

- Analysis on the interaction between bacteria leading to cystic fibrosis
- Preparing necessary bacterial cultures
- Executing bioluminescence experiments
- Prepare all necessary standards
- Analysis of data and deviations

ACTIVITIES
Volunteer at Lodi Ambulance and Rescue Squad, Nov. 2007-2010

LANGUAGES
Trilingual: English/Turkish/Kurdish