

SOAR Proposal - Summer 2016

Manipulating Signal Hydrophobicity to Alter Quorum Sensing in *Streptococcus pneumoniae*

Faculty Mentor: Michael A. Bertucci, Assistant Professor of Chemistry

Student Researcher: Erin Tiwold

Start Date: May 31st, 2016

Length of Project: 10 weeks

Project Summary

Bacteria utilize a process known as quorum sensing to communicate with each other to coordinate group gene expression based on the number of neighboring cells. During quorum sensing, bacteria produce a signaling molecule that is released outside the organism. As the density of bacteria increases so does the amount of signaling molecules. At a certain concentration these molecules allow the initiation of group gene expression resulting in processes such as bioluminescence, biofilm formation, and host infection. Bacteria utilize quorum sensing to communicate because it is advantageous to initiate their mechanism of infection when the concentration of bacteria is high, potentially overriding a host immune system that can initiate an attack and wipe out the population.¹

Bacteria are becoming resistant to many antibiotics on the market that are currently used to treat such infections since antibiotics lead to bacterial cell death. This then causes selection for mutants that are resistant to the medication prescribed. This is why it is applicable to find a way to inhibit the way bacteria communicate to each other. Such an anti-infection strategy will not wipe out the bacteria population but create an environment where the bacteria believe they are present at low density and will not initiate gene expression.²

We want to study the quorum sensing mechanism used in *Streptococcus pneumoniae*. This pathogen is responsible for bacterial pneumonia, causing more than 1.2 million infections per year. *Streptococcus pneumoniae* is an ideal bacterium to study since the signaling molecule (a peptide known as CSP1) has already been identified. We will employ synthetic organic chemistry to make CSP1 derivatives, focusing on modification at a uniquely hydrophobic region of the peptide that is hypothesized to be critical for receptor binding and initiating gene expression.³ These derivatives will not only allow us to study how the peptide binds to the receptor, but may also serve as inhibitors of the native peptide, CSP1. This would decrease the efficiency of the bacteria to communicate with one another and attenuate virulence. We will determine this experimentally by screening our synthetic signaling molecules in live *streptococcus pneumoniae* cultures with a collaborator at the University of Nevada, Reno at the end of August.

Roles & Responsibilities

I, Dr. Bertucci, will serve as a mentor for all 10 weeks of the proposed project providing one-on-one instruction to the student, Erin, in peptide synthesis, purification, and analysis. I will also be responsible for supporting Erin in her search and analysis of peer-reviewed literature related to our project and providing feedback on Erin's progress and results in daily individual meetings. Further, I will be in charge of procuring the supplies Erin needs to complete her proposed project and arrange registration and travel for her presentations and potential meetings with collaborators.

¹Bassler BL. How bacteria "talk". TED; 2009 Feb [cited 2016 Mar 10]. Available from: http://www.ted.com/talks/bonnie_bassler_on_how_bacteria_communicate

²Rutherford ST, Bassler BL. Cold Spring Harb Perspect Med. 2012 Nov 1;2(11):a012427.

³Tian, X., Syvitski, R. T., Liu, T., Livingstone, N., Jakeman, D. L., Li, Y. H., Biological Procedures Online. 2009; 11(1).

Erin will be the lead researcher on the project responsible for carrying out all experiments towards our research goals. She will document her experiments in an approved laboratory notebook. At the end of her SOAR experience, Erin will compile all of her chemical products, analytical data, and relevant procedural documentation for reference by future students and for publication. Erin will participate in daily individual research meetings with her mentor and formally present her progress to her labmates and mentor in the form of a powerpoint presentation at least 3 times throughout the summer. As a summative activity, Erin will present her work at the National Meeting of the American Chemical Society and Moravian College's Undergraduate Student Scholarship and Creative Arts Day.

Timetable of Expected Milestones

WEEK 1: Orientation to literature, project goals, and experimental procedures; design of CSP1 derivatives

WEEK 2 - 4: Synthesis of first class of CSP1 derivatives

WEEK 5 - 7: Purification and identification of first class of CSP1 derivatives; synthesis of second class of CSP1 derivatives

WEEK 8 - 10: Purification and identification of second class CSP1 derivatives; poster preparation for American Chemical Society National Meeting

Engagement in Discipline-Specific Research

This project will allow Erin to engage in discipline-appropriate scholarly research as a biochemistry student. She will be able to engage in cutting edge research where chemical modification to the quorum sensing communication of the selected organism has not been heavily researched. This will allow her to make new discoveries that will relate to the field of biochemistry and medicine, her current and future fields of study. It will further her knowledge on content, techniques in a lab, and strengthen critical thinking and problem solving skills. Erin will learn how to adapt the project based on data and how to interpret findings. This opportunity will allow her to experience how to conduct independent research and how to adapt to achieve research goals.

Contributions to the Discipline & Community

Erin's project is centered on understanding and manipulating a chemical communication pathway in one of the world's most prevalent bacterial pathogens. Despite the notable scientists working on bacterial quorum sensing, no one has yet developed successful peptide-based inhibitors for *Streptococcus pneumoniae*. My expectation is that Erin's work this summer will contribute to the body of work my collaborator at Nevada, Reno and I have begun to build and be included in a future peer-reviewed publication.

Erin will have the opportunity to present the project at the National Meeting of the American Chemical Society in August in Philadelphia, PA. She will also present her findings to the college community at the Undergraduate Student Scholarship and Creative Arts Day in the Spring of 2017. I am also currently working with my collaborator to secure funding to bring Erin out to University of Nevada, Reno to run the bacterial assays herself! Not only would she help with a critical step in our collaboration, but she would have the opportunity to network and experience life in a larger academic research environment.

Student Statement of Purpose

Manipulating Signal Hydrophobicity to Alter Quorum Sensing in *Streptococcus pneumoniae*

Student: Erin Tiwold

Major: Biochemistry

Expected date of graduation: December 2016

Faculty Mentor: Dr. Michael Bertucci

Request for on-campus housing: Yes

I am seeking to conduct research through the SOAR program because I am passionate about the sciences and learning. I have taken many courses in biology, chemistry and as an upperclassman I have had the opportunity to take courses like biochemistry and advanced genetics that puts it all together. Through my courses I have found that biochemistry is my favorite subject because it demonstrates the intricacy of how molecules interact with others to perform a function.

As a junior biochemistry major and field hockey player I have developed a strong work ethic, determination, time management, and teamwork skills that I will utilize during this project. I always give everything my best effort and I am a quick learner. I am passionate about the sciences, life long learning, and challenging myself to conduct research which is ultimately why I am pursuing a SOAR project.

Dr. Bertucci's project interests me because it involves the investigation on how bacteria use quorum sensing to communicate to other bacterial cells in order to initiate group gene expression. When bacteria initiate virulence, the organism, for example humans can become sick. Currently we use antibiotics to treat bacterial infections however more and more antibiotics are becoming ineffective due to bacterial resistance. This is something that in my future as a physician I will most likely deal with as drugs that I would normally prescribe for an infection may or may not be effective due to the strain of bacteria infecting the individual. Therefore research to find a different way to prevent bacterial virulence will hopefully lead to a new method of treatment one day.

This project is also important to further my education because it will allow me to grow as an undergraduate student. Conducting research for 10 weeks will enrich my education by allowing me to pursue a single project in duration. Implementing a lab for three hours at a time has taught me many techniques and given me the ability to analyze data which is necessary however the inability to pursue a project for weeks does not allow for one to experience the missteps and the process of trying to fix the problem. This project will also help me learn how to work in collaboration with a professor as well as work individually as a student in solving a more complex, detailed, scientific problem.

At the end of the SOAR project I expect to have learned about a topic in great detail as well as learn as much as I can from my faculty mentor. I want to fulfill as many goals possible in a 10 week time span. I plan to learn and execute how to synthesize derivatives of the signaling molecule, purify, and analyze the derivatives. I plan to put together a poster presentation and at the end of the project I plan to have the opportunity to present my work with the Moravian College community and at the National Meeting of the American Chemical Society in August in Philadelphia, PA.

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Expense Proposal

Towards the completion of the research and experiences discussed above, we are requesting funding for the following expenses:

ACS Membership Registration:	\$27.00
ACS National Meeting Registration:	\$105.00
Fmoc-Lys(Boc)-OH (500 g)	\$370.00
Total	\$502.00

The ACS Membership Registration Fee will allow Erin to become a student member of the American Chemical Society and receive a reduced registration fee for the National Meeting at which we plan to present her research. The ACS National Meeting Registration is that fee.

Fmoc-Lys(Boc)-OH is a chemical that is required for the preparation of Erin's CSP1 derivatives. We do not have this chemical present in the chemistry department at Moravian and it will be used exclusively for research purposes.