

## SOAR Research Proposal - Summer of 2017

### Effects of modifying of carbon number and structure of hydrophobic amino acids in CSP-1, a key quorum sensing peptide in *S. pneumoniae*.

**Faculty:** Dr. Michael Bertucci, Assistant Professor of Chemistry

**Student:** Robert Hillman, Department of Chemistry, Class of 2018

**Project Start Date:** May 29, 2017

**Length of Project:** 10 weeks

#### Project Description:

Bacterial resistance to antibiotics is becoming one of the most difficult to address problems facing the modern medical community. Infections by pathogenic bacteria are now more difficult to treat than they have been in decades due to the rate at which bacteria are gaining resistance to even the most potent antibiotic drugs. Bacterial quorum sensing is one important cellular process in bacterial resistance, and the ability to modify this process has the potential to aid in slowing or even stopping the rate at which bacteria can become resistant.

Quorum sensing<sup>1</sup> in bacteria is similar to having a quorum call in Congress; only when enough members are present can any decisions be made. Bacteria release small molecules or short proteins (known as peptides) in order to communicate with other cells of the same species. As the number of bacterial cells in an area in a host increases, so too does the concentration of these signaling molecules. When the bacteria reach a “quorum” the concentration of the chemical messenger is high enough around the cells to cause them to respond to the message. This has multiple implications in the pathogenicity of bacteria and the cells’ resistance.

This research project will specifically focus on quorum sensing in the human pathogen *Streptococcus pneumoniae*. *S. pneumoniae* is responsible for bacterial pneumonia, bacterial meningitis, and inner ear infections. The chemical messenger in question with this bacterium is known as Competence Stimulating Peptide 1 (CSP-1), which is a 17-amino acid peptide chain that *S. pneumoniae* uses to communicate.<sup>2</sup> The process regulated by CSP-1 is called competence. From a bacterial standpoint, competence is the ability of a cell to pick up fragments of nucleic acids from the environment and insert it into the cell’s own genome. There are several types of bacterial antibiotic resistances that can be spread through competence, therefore controlling this process could have serious medical benefits in the treatment of infections.<sup>2</sup>

The goal of the project is to synthesize derivatives of CSP-1 in the lab using the method of solid phase peptide synthesis and screen them for quorum sensing activation and inhibition in live bacterial assays. These derivatives will contain alterations at the hydrophobic (water-repelling) sites of CSP-1 that have been implicated in receptor binding. Amino acids only varying in the number of carbon atoms relative to the natural amino acids will be substituted. As a result, six novel CSP-1 derivatives will be synthesized for testing in biological assays. After the peptides have been prepared, they will be isolated and purified. The pure CSP-1 derivatives will then be taken to the University of Nevada, Reno to be screened in biological assays with *S. pneumoniae* to test whether they can compete with the natural peptide to bind to the native cell receptor.

#### Roles and Responsibilities:

I, Dr. Bertucci, will serve as Robert’s direct mentor during 7 weeks of his proposed project, while my collaborator (Dr. Yftah Tal-Gan) at UNR will be his direct mentor in the final 3 weeks. I will be in contact with Robert each day (via phone and/or email) to advise him during his time in Nevada. I will be responsible for monitoring Robert’s

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<sup>1</sup> C.M. Waters, B.L. Bassler. “Quorum Sensing: Cell to Cell Communication in Bacteria.” *The Annual Review of Cell and Developmental Biology*, 21, 319-346 (2005)

<sup>2</sup> L. Zhu, G.W. Lau. “Inhibition of Competence Development, Horizontal gene transfer and virulence in *Streptococcus Pneumoniae* by a Modified Competence Stimulating Peptide.” *PLOS Pathogens*. 7, 1-15 (2011).

progress, working with him one-on-one as he explores academic literature and formulates his experiments, and providing consultation on learning new techniques and interpreting data. In addition, I will gather the supplies and chemicals Robert needs to carry out his proposed research and arrange travel to and lodging in Nevada and at the conferences at which Robert will present his results.

Robert will be the individual conducting all the research and experiments to forward his project goals. As common in our discipline, Robert will be responsible for maintaining a laboratory notebook to document his results and progress. At the end of the summer, Robert will compile all of the peptides that he has synthesized, the biological data he has acquired, and other relevant documentation in an organized fashion for reference by future students and publication. Robert will participate in daily research meetings with Dr. Bertucci and any other students being advised by Dr. Bertucci to set a daily research agenda and discuss results from the previous day's work. As summative evaluations of his progress and opportunities for academic and professional development, Robert will present his work at the Moravian College Undergraduate Student Scholarship and Creative Arts Day, the University of Nevada, Reno, and the National Meeting of the American Chemical Society in Washington, D.C.

**Project Timetable:**

WEEK 1 & 2: Synthesis of first set of CSP-1 derivatives (3 peptides)

WEEK 3 - 5: Purification of first set of CSP-1 derivatives; Synthesis of remaining CSP-1 derivatives (3 peptides)

WEEK 5 - 7: Purification of second set of CSP-1 derivatives; Synthesis & purification of doubly substituted derivatives

WEEK 8 - 10: Completion of *S.pneumoniae* bioassays at the University of Nevada, Reno

**Qualifications of the Student:** Robert has completed organic chemistry I & II as well as an upper-level course in bioorganic & medicinal chemistry, which is directly relevant to the research contained in our SOAR proposal. He excelled in all three courses and therefore has the necessary background to understand and carry out his project. I have already been working with Robert as part of an independent study. Through this, I have been very impressed with how Robert has conducted himself and I have full confidence in his ability to achieve his proposed research goals. In addition, he has gained extra laboratory skills working as a teaching assistant for organic chemistry II this semester. As a junior chemistry major who currently plans to continue on to graduate school, I know this would be a valuable experience in helping him achieve his future goals.

**Student engagement in discipline-appropriate scholarly research:**

Peptide synthesis fits in a field blended between biochemistry and organic chemistry, appropriately called bioorganic chemistry. During this project, Robert will use many of the synthesis and purification techniques common in this field. This will be an invaluable experience to him as a chemistry major with declared interest in bioorganic and medicinal research. The planning and execution of each stage of this research project will be done with the aid of information gathered from peer-reviewed literature. Robert will use this information in addition to the data he has gathered and his lessons learned from his coursework to assess the impact of his CSP-1 derivatives on the *S.pneumoniae* quorum sensing network. In addition, Robert will have the opportunity to complete the biological assays on the derivatives he synthesizes at the University of Nevada, Reno. This will be a unique educational experience in both the discipline appropriate techniques he will learn and the three weeks he will spend with post-doctoral and graduate students in an R1 laboratory.

**Contributions to the Discipline and Opportunities to Share Work:**

This research involving CSP-1 derivatives will offer insight into the way in which the natural peptide binds in the receptor site in *S. pneumoniae*. This is key to its activity in the cell. This has the potential to lead to an understanding of a way to block this pathway in *S. pneumoniae* and other bacteria, also. Despite all the research being conducted on quorum sensing, no one has yet developed successful peptide-based inhibitors for *Streptococcus pneumoniae*. Thus, Robert will obtain novel data through the proposed project that 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. Robert will play a key part in maintaining this collaboration, which was just initiated last year.

Robert will have the opportunity to share his work at the Moravian College Undergraduate Student Scholarship and Creative Arts Day, the University of Nevada, Reno, and the National Meeting of the American Chemistry Society in Washington, D.C.

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**Student:** Robert Hillman

**Major:** Chemistry

**Expected Date of Graduation:** May, 2018

**On Campus Housing:** No

**Statement of Purpose:**

As a chemistry major with a specific interest in organic chemistry and synthetic chemistry, this particular project is very interesting to me. The ability to create novel compounds in the lab is an exciting prospect, and is something that I wish to pursue both in graduate school and in my later career. SOAR offers a perfect opportunity for me to be able to better focus on the research that I plan to do, more than I can during the academic semester. The project also allows me to apply the theory I learned in the Bioorganic and Medicinal chemistry course I took last fall to a concrete chemical environment that can be analyzed instead of just seeing it on a page in a textbook. I believe a SOAR project will also show me whether or not I am truly interested in pursuing this type of chemical research in the future. This project will be very beneficial to me both academically at Moravian and in the future when applying to graduate schools.

I hope to gain knowledge and experience working in an organic chemistry lab, as well as in using the instruments that are necessary to the project. This experience will be extremely valuable to my future career. I intend to learn how to safely and effectively perform synthetic reactions in a laboratory setting through my SOAR experience. I also want to more deeply research the biological organic chemistry I have learned in class and learn it better by actually doing it. I believe that taking part in this project will make me a better student all around, as well as a better chemist. I also feel that it will offer me an insight into the future that I can have in the field as I continue my studies in chemistry and move on into a job in the chemical industry. This project will greatly increase my chances of success in many of these areas by offering concrete experiences in a way that a normal lecture-based course cannot.

I have also always wanted to be part of the medical field. There is nothing that I have been more passionate about, and while I do not see myself as a medical doctor, I can see myself being a medicinal chemist. This project is a perfect opportunity for me to learn and research a very important aspect of the modern medical field, as well as chemistry: biologic drugs. As scientists and doctors search for better, more targeted approaches to treat diseases it is increasingly common for the new drugs that come to market to be these so-called biologics. These are drugs that mimic natural molecules found in the body such as small proteins and nucleic acids. These biologic drugs have offered progress in diseases that have not seen successful treatments with any other class of drugs available so far. Those with cancer, rheumatoid arthritis, drug-resistant infections, and even muscular dystrophy have been given hope with this new wave of drugs. This project is looking at exactly this type of research. This opportunity with SOAR is in exactly the field I want to join, but I always thought that I would have to wait until graduate school to do this work. I am excited for this chance to research a topic in my field of interest.

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**Student:** Robert Hillman

**Major:** Chemistry

**Expected Date of Graduation:** May, 2018

**Expense Proposal:**

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

Fmoc-HomoPhe-OH (5 g):	\$82.80 + shipping
Fmoc-Nle-OH (5 g):	\$25.00 + shipping
Fmoc-Phg-OH (5 g):	\$20.70 + shipping
<u>Chemglass 25-mL Peptide Vessel (Quantity 2)</u>	<u>\$341.74 + shipping</u>
<b>Total:</b>	<b>\$470.24 + shipping</b>

Fmoc-HomoPhe-OH, Fmoc-Nle-OH, and Fmoc-Phg-OH are chemicals that are required for the preparation of Robert's CSP-1 derivatives. The 25-mL Peptide Vessels will be critical for Robert to carry out the reactions to form his peptides. We do not have these chemicals and equipment present in the chemistry department at Moravian and it will be used exclusively for research purposes.