

Communicating Research to the General Public

At the March 5, 2010 UW-Madison Chemistry Department Colloquium, Prof. Bassam Z. Shakhashiri, the director of the Wisconsin Initiative for Science Literacy (WISL), encouraged all UW-Madison chemistry Ph.D. candidates to include a chapter in their Ph.D. thesis communicating their research to non-specialists. The goal is to explain the candidate's scholarly research and its significance to a wider audience that includes family members, friends, civic groups, newspaper reporters, program officers at appropriate funding agencies, state legislators, and members of the U.S. Congress.

Over 20 Ph.D. degree recipients have successfully completed their theses and included such a chapter.

WISL encourages the inclusion of such chapters in all Ph.D. theses everywhere through the cooperation of Ph.D. candidates and their mentors. WISL is now offering additional awards of \$250 for UW-Madison chemistry Ph.D. candidates.



The dual mission of the Wisconsin Initiative for Science Literacy is to promote literacy in science, mathematics and technology among the general public and to attract future generations to careers in research, teaching and public service.

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May 2014

Time-lapse fluorescence microscopy of the effects of antimicrobial peptides on single, live *Escherichia coli* cells

By

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A dissertation submitted in partial fulfillment of
the requirements for the degree of

**Doctor of Philosophy
(Chemistry)**

at the

UNIVERSITY OF WISCONSIN MADISON

2015

Date of final oral examination: 08/24/2015

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There's no better learning than that acquired via questions and answers. Through a Q&A format, this chapter aims to provide a brief overview of my graduate work to the general public. I am grateful to the Wisconsin Initiative for Science Literacy for giving me this opportunity to connect with a broader audience.

Q1. Can you describe the aim of your thesis in one sentence?

In my graduate work, I have developed a detailed understanding of how a particular class of antibiotics (Antimicrobial Peptides or AMPs) kills live bacterial cells. (Peptides are small fragments of proteins present in living organisms)

Q2. What are antibiotics and why do we need to study them?

Think of the last time you were running a temperature and visited a clinic. Part of the diagnosis would have been to figure out whether you had a bacterial infection. If so, the doctor would have prescribed an antibiotic dose for 3-7 days.

Antibiotics are “wonder drugs” that have been used to treat bacterial infections since the 1940s. Penicillin, arguably the most popular antibiotic, was serendipitously discovered in 1928 by Alexander Fleming, a Professor of Bacteriology at St. Mary’s Hospital in London. On returning from a vacation, Fleming was sorting bacterial samples, which were streaked out on petri dishes containing bacterial food. He noticed that one of the petri dishes had a mold growing on it, and the zone immediately surrounding the mold was cleared of all bacterial growth. Apparently, some substance secreted by the mold had inhibited bacterial growth in its vicinity.

Subsequent research performed by Howard Florey, Ernst Chain and their colleagues at Oxford University converted this research curiosity into a life-saving drug!

Over the past seventy years, several new antibiotics have been introduced in clinical use. These have undoubtedly contributed to global health and well-being. However, prolonged and excessive use of antibiotics has allowed disease-causing bacteria to adapt in several ways. These ‘improved’ bacterial strains are now able to resist the same antibiotic treatments. Infections caused by ‘antibiotic resistant’ bacteria are one of the leading causes of sickness and death today. The CDC (Centers for Disease Control and Prevention) estimated that such bacteria caused more than 2 million infections in 2013 in the United States, of which 20,000 cases were fatal. Some prominent pathogenic bacteria are MRSA (Methicillin Resistant *Staphylococcus Aureus*), *C. Diff. (Clostridium Difficile)* and *Acinetobacter*. Of particular concern to scientists and doctors are bacteria that resist all known antibiotic treatments – these ‘superbugs’ are virtually impossible to kill.

There is a constant tug-of-war between bacteria and antibiotics being played out in our hospitals and health-care facilities. Quite frankly, the bacteria are winning this hands down! In several cases, within only a few years of a drug being introduced in the market, bacteria are able to adapt and render it ineffective. To add to the problem, the FDA has not approved any new families of antibiotics over the last two decades.

Q3. Why are Antimicrobial Peptides (AMPs) important?

As is clear from the answer to Q2, there is a pressing need for novel, potent antibiotics that work against all kinds of bacteria. This is where several research groups have proposed a

crucial role for Antimicrobial peptides (AMPs). AMPs are part of our immune systems – they are present on our skin, in our saliva, tears and in white blood cells. AMPs help us fight infections by directly killing invading bacteria, and by signaling our immune systems to start working! They were first discovered in the 1970s. Scientists have discovered more than 2500 natural AMPs with varying structures, lengths and sequences. Some AMPs are shown in Figure 1. Almost every organism has one of these ‘natural’ antibiotics.

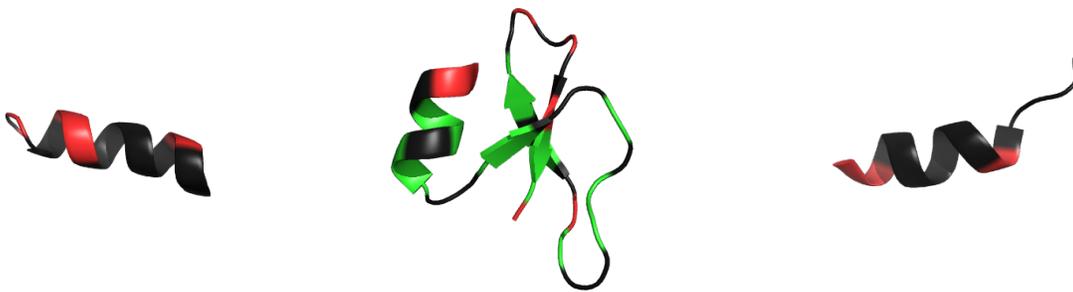


Figure 1: Structures of various AMPs. From left to right, CM-15 (hybrid, synthetic AMP), Human Beta Defensin-1 (human AMP) and Indolicidin (bovine AMP). The colors represent amino acid residues that are hydrophobic, polar or charged.

Conventional antibiotics bind to specific target molecules on the bacterial cell surface. Resistant bacteria have evolved to produce a slightly different version of these target molecules that does not interact with antibiotics. This way, drugs cannot interact with bacteria and are, therefore, rendered ineffective. AMPs evade bacterial resistance because they do not have a specific target molecule on the cell surface. Instead, they bind generally to the cell wall, which consists of a mixture of layers of fats and sugar molecules. Evidently, modifying the composition of an entire cell wall is more challenging for bacteria than changing a single target molecule. Due to this, AMPs are considered as potential candidates for developing the next generation of antibiotics. Research on AMPs has accelerated over the past two or three decades. Currently,

there are approximately ten AMPs in various stages of clinical trials. To improve our understanding of how AMPs work, the Weisshaar group observes their action on live bacteria using high-end scientific microscopes and related techniques.

Q4. Which type of bacteria did you study?

In my experiments, I studied the model bacterium *Escherichia coli* (*E. coli*). Our group has worked with this bacterium for several years. It is one of the most well understood species of bacteria. It was recently in the news due to an outbreak of the pathogenic strain *E. coli* O157:H7. Thankfully (!), I worked with a non-pathogenic laboratory strain, *E. coli* MG1655 and its variants. The *E. coli* cell is rod-shaped with a length approximately 1/50th the diameter of a human hair.

Q5. What are the major findings of your work?

We observed that AMPs kill bacteria by permeabilizing (i.e. by making permeable) their cell membranes and halting cell growth. The AMPs preferably targeted actively dividing cells over non-dividing cells. We also observed that the membranes were permeabilized in very specific regions – close to the division plane where daughter cells pinch off from each other, and near the cell poles (tips of the cell). The effects of AMPs were persistent, and once the membranes are permeabilized, cells do not recover even after the AMPs were rinsed out.

We studied AMPs that are naturally present in humans and insects (moths). We also worked with synthetic peptide analogs that were engineered to mimic natural AMPs. In our experience, each AMP causes a unique set of antimicrobial events in bacteria. There is lot of

variation in the cellular responses. Using our powerful assays, we observed these various events in real time and developed a detailed understanding of antimicrobial action.

All AMPs studied in this work permeabilized the cell membranes. Some AMPs also caused secondary effects including disruption the way DNA is wound up inside the cell. We are slowly beginning to understand that AMPs might be targeting multiple cellular processes simultaneously, which could explain why bacteria find it harder to outsmart AMPs than conventional antibiotics, which typically have a single target.

Q6. What method did you use for your experiments?

We used live-cell videography to observe the effects of AMPs on single *E. coli* cells. To characterize AMP action, we developed various assays to study membrane permeabilization, effect on cell growth, and perturbation of intracellular molecules such as the DNA.

Q7. What is the advantage of using the method over others?

Several other groups study AMP action on bacteria. However, most of them use methods that give an output signal that is averaged over all the cells in the tube (likely millions or billions of cells). This way, the diverse behavior of the various cells is blurred out. By now, we know that no two cells are identical, even those that have the same DNA! Therefore, it is likely that no two cells behave in an identical manner. Scientists believe that this cell-to-cell variation may be crucial to our understanding of how diseases such as cancer progress over time. Therefore, it is

crucial to develop methods to watch individual cells. Our single-cell videography methods provide this advantage over other techniques.

Although many research groups use such single-cell imaging approaches, only a handful of them study antimicrobial action. To our knowledge, there are less than ten laboratories in the world that are studying AMPs using single-cell imaging.

Q8. What kind of microscope did you use?

Our research group assembled a microscope in-house over several years. We purchased most of the parts from various companies and built some accessories in a machine shop in our department. Briefly, the instrument consists of the following parts:

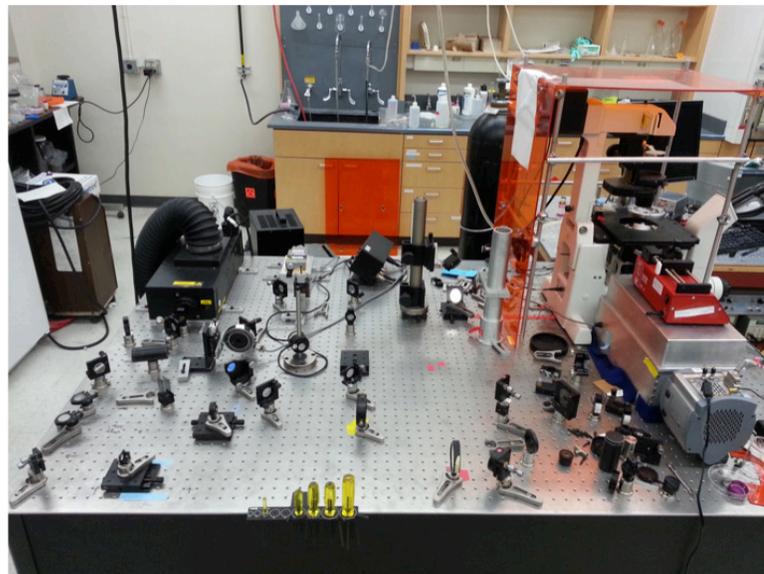


Figure 2: A picture of the microscope setup used in this thesis. In the foreground, several mirrors and lenses are arranged to direct laser beams onto the sample. The black box on the table (top left) is a laser. The eye-piece and microscope stage can be seen on top right. The gray box close to bottom right is a high-end camera.

Microscope: Part of the instrument looks similar to a table-top microscope that you may have used in high school biology laboratories. However, our setup was equipped with an optics table (Figure 2) that consists of a maze of mirrors, lenses, shutters, lasers... even a periscope! All these widgets help to shape the path of laser beams onto the sample. The microscope is fitted with objective lenses that we selected based on their light-bending properties. Various terms used to describe details of these lenses are similar to those used for modern digital cameras (such as numerical aperture, working distance and depth of field). The whole table is floating on Nitrogen gas! This insulates the optics from any vibrations that get transmitted through the walls or floor of the building.

Camera: Our microscope is fitted with a high-end, ultra-sensitive scientific camera designed specifically to acquire images at a very high frame rate. It is similar to the latest point-and-shoot cameras that you may have used to click photos, only much faster and more sensitive. The camera is connected to a computer, and operated by computer software. We can save images and movies, to be watched and analyzed later.

Flow chamber. We plate live bacteria on a glass slide. The slide is part of a small chamber into which we flow solutions containing ‘bacterial food’! We can also control the temperature of the solutions and chamber. Together, this helps keep the bacteria alive and happy. When it is time to begin the experiment, we start flowing a solution of the AMP and watch how cells respond.

Q9. Can AMPs become marketable drugs one day?

This remains to be seen. Currently, no AMPs are being used clinically. (Colistin, also known as Polymyxin E, is the closest candidate. It is a peptide-based antibiotic that has been used to treat certain bacterial infections. However, a major part of its structure is made up of lipids, so it is not included in the larger family of AMPs). There are about ten AMP candidates in various stages of clinical trials. However, more research is needed to understand the detailed effects of AMPs. We do not know how AMPs permeabilize membranes, how many AMP molecules are needed to kill a bacterial cell, how bacteria could develop resistance to AMPs, and how the structure of AMPs influences their function. Research geared toward answering these and several other open questions will undoubtedly contribute to the development of potent, AMP-based antibiotics.

Q10. Overall, how would you describe your graduate research experience?

Graduate research has been fun and challenging. I really enjoyed the basic experimental procedure in the lab - observing live cells, shooting movies, playing the movies back, analyzing them and trying to make sense of the data. To me, this was a great way to spend my work day! Making sustained progress in my research was a lot more difficult than I had initially imagined. Living cells are inherently diverse, and give varied responses under the same set of conditions. Therefore, being able to reproduce observed trends was quite challenging. I was fortunate to get great mentorship from my advisor, and a lot of help from my colleagues in the group. Useful collaborations with other research groups also significantly contributed to the progress that I made on my project.

Overall, graduate research has been an inspiring, rewarding experience!

As part of the post-defense celebrations, my wife Rika and my friends surprised me with their brilliant creativity on this cake! You can see a 'teary-eyed' bacterial cell in the middle of the cake, fearing its impending doom by the red AMP molecules. Yours truly is rocking the tassel and commanding the AMPs to their mission! The AMP in 'I am Ph.D.' stands out, as it should ☺

