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 40 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.

Wisconsin Initiative for Science Literacy

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.

UW-Madison Department of Chemistry
1101 University Avenue
Madison, WI 53706-1396
Contact: Prof. Bassam Z. Shakhashiri
bassam@chem.wisc.edu
www.scifun.org

Single-Cell, Real-Time Detection of Antimicrobial Peptide's Attack in Live *E. coli* Cells

By

Zhilin Yang

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The dissertation is approved by the following members of the Final Oral Committee:

James C. Weisshaar, Professor, Chemistry

Lloyd M Smith, Professor, Chemistry

Samuel H. Gellman, Professor, Chemistry

Alessandro Senes, Associate Professor, Biochemistry

Chapter 6

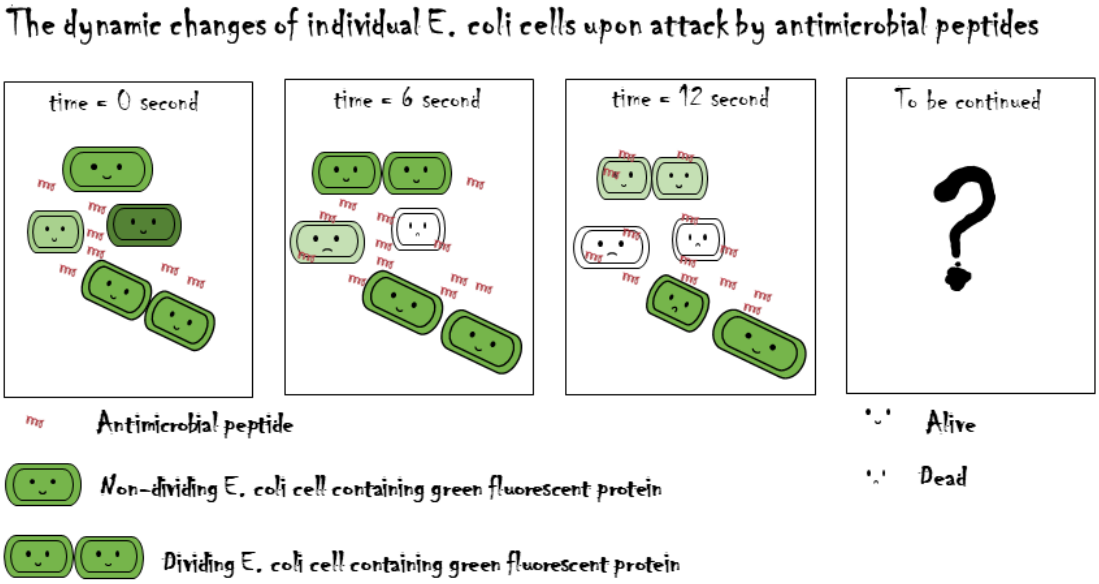
Track antimicrobial peptides actions on single *E. coli* cells under the microscope

Prepared for the WISL Award for Communicating Graduate Chemistry Research to the Public.

Thanks to Professor Bassam Z. Shakhashiri for the event initiation and organization. Many credits to Professor Bassam Z. Shakhashiri, editor and Cayce J. Osborne for the comments.

Q1: Can you briefly describe your research?

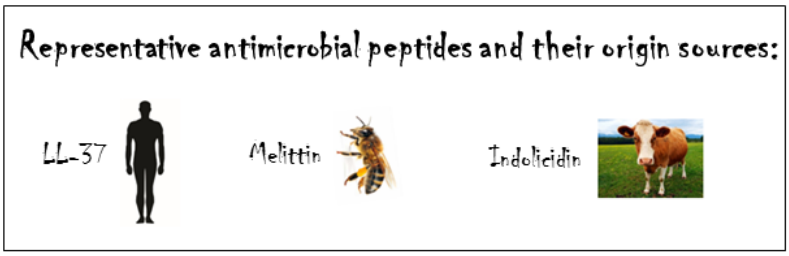
I use a fluorescence microscope (a microscope that uses fluorescence to generate an image) to track responses of individual *E. coli* (a common bacterium, refer to Q4 for more information) cells upon exposure to antimicrobial peptides (a special type of antibiotics, defined in Q2). In the example below, the green color represents a kind of protein which fluoresces green after exposure to a laser. Darker green contains more green fluorescent protein. At the beginning, the four cells all contain some green fluorescent protein. However, their experiences upon attack by antimicrobial peptides are completely different. For example, some cells become longer initially but shorter later, partially losing green fluorescent protein. Some cells die immediately and become shorter and lose green fluorescent protein completely.



Q2: What are antimicrobial peptides and where do they come from?

Antimicrobial peptides are relatively small peptides (peptides are compounds consisting of two or more amino acids linked in a chain) that protect a host from bacterial infection, by either modulating host immunity or killing bacterial cells. Antimicrobial peptides act on bacterial cells in many ways. For example, they can disrupt bacterial membranes, resulting in leakage from the cell, or they can mess up bacterial intracellular metabolism activities. All these actions can lead bacterial cells to stop growing or even die.

Antimicrobial peptides can be found nearly everywhere in nature. For example, the antimicrobial peptides studied in my thesis work include LL-37 that is found under our skin, Melittin originating from bee venom, and Indolicidin coming from cows. These antimicrobial peptides all have different structures and conformations. So far, almost 3000 natural antimicrobial peptides have been discovered.

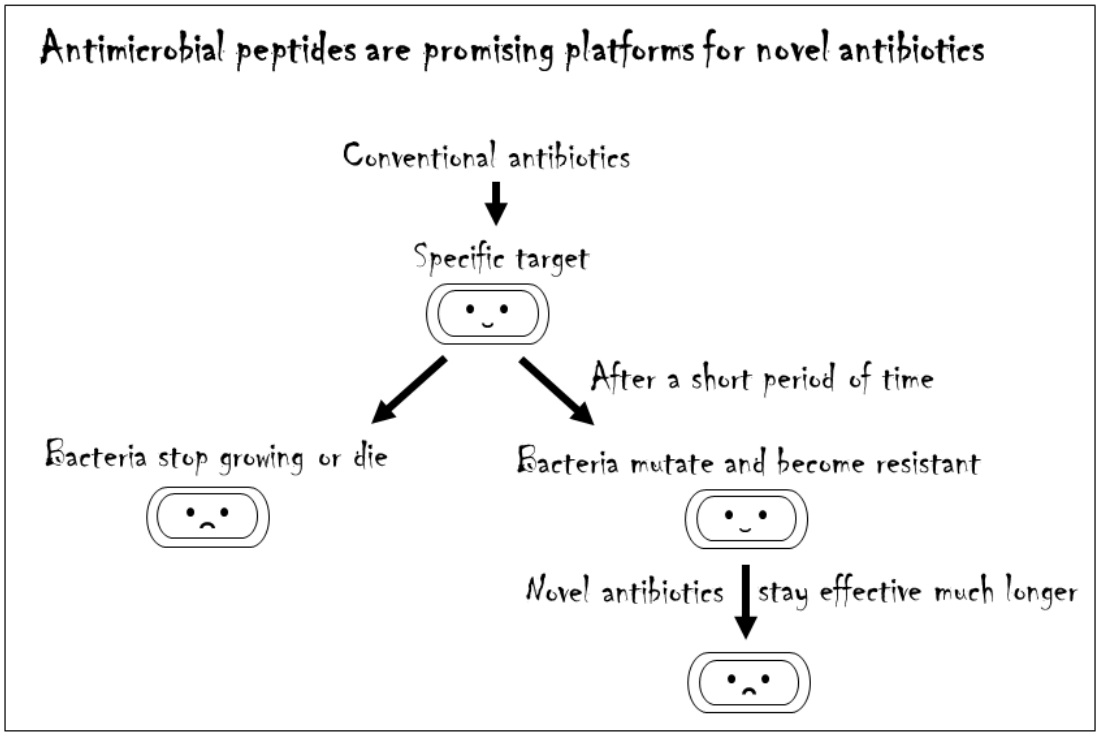


Q3: What is the significance of studying antimicrobial peptides?

A healthy body keeps good bacteria but not harmful bacteria. If we have either a low level of good bacteria or a high level of harmful bacteria, we are likely to suffer from many chronic inflammatory diseases, such as asthma, cancer, and stroke. Studies have shown that antimicrobial peptides contribute to the proper maintenance of microorganisms in our bodies.

Exploring the actions of antimicrobial peptides on bacterial cells can help us understand why we are able to but sometimes fail to maintain the appropriate level of good bacteria and harmful bacteria.

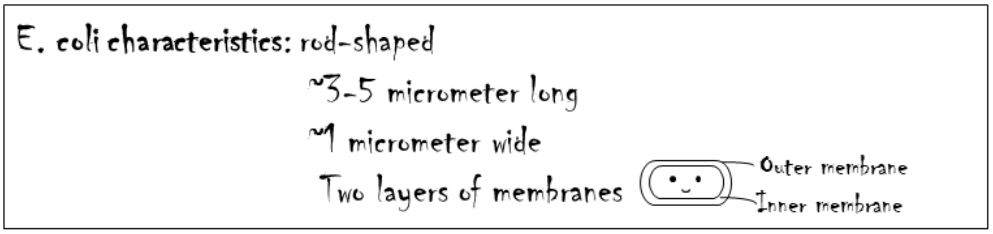
Additionally, antimicrobial peptides are considered promising platforms for novel antibiotics. As you might know, we are currently facing a severe antibiotic crisis. For some bacteria-caused diseases, the current commercially available antibiotics are no longer effective.



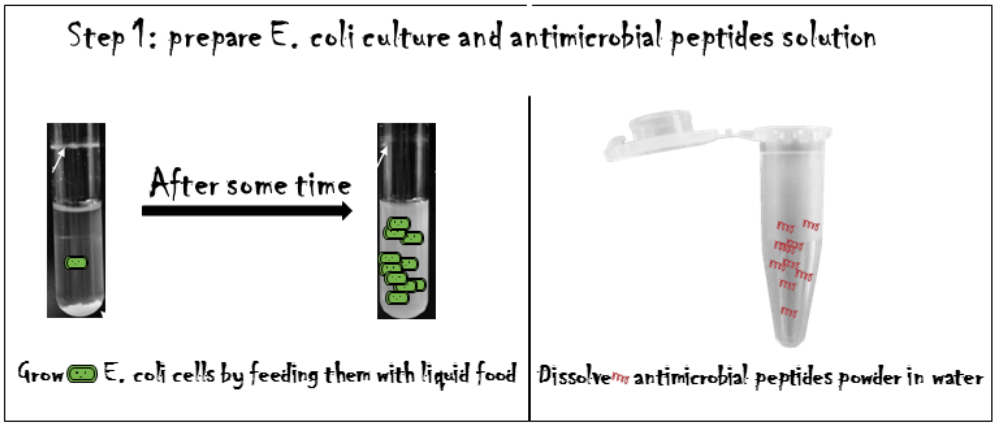
The major reason why this is happening is that conventional antibiotics only have specific targets within the bacteria. Once bacteria mutate those specific targets, the conventional antibiotics become ineffective. In contrast, antimicrobial peptides target bacteria in many different ways as mentioned in Q2. Therefore, it takes much longer for bacteria to mutate and escape from all the actions of the antimicrobial peptides.

Q4: What bacterial system are you studying?

We chose *E. coli* (*Escherichia coli*) as our model system, as it is a very common and well-studied bacterium. *E. coli* normally live in the intestines of people and animals. Most *E. coli* are harmless and actually are an important part of a healthy human intestinal tract. However, some *E. coli* are pathogenic, meaning they can cause illness such as diarrhea. The pathogenic *E. coli* in general come from contaminated food or water. *E. coli* consists of many species, and our lab started with a non-pathogenic one called MG1655. The common characteristics of *E. coli* are listed in the graphic below.



Q5: How do you perform the experiments?



Step 2: Set up lasers and microscope

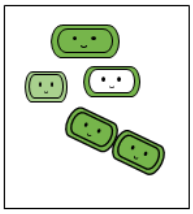
A table equipped with different-wavelength lasers and various optics that direct laser beams to the microscope

A microscope attached to camera and computer

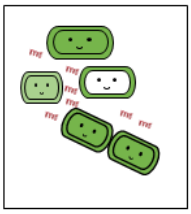
Step 3: Add antimicrobial peptides solution to E. coli cells in a small chamber

A small chamber placed on microscope, providing ~10 microliter space

(a) Flow E. coli cells to a small chamber

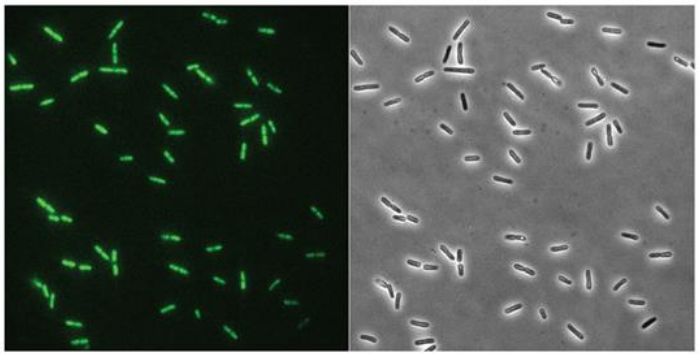


(b) Flow antimicrobial peptides solution



E. coli cells are immobilized but stay alive in the small chamber

Step 4: Take movies via software installed in computer

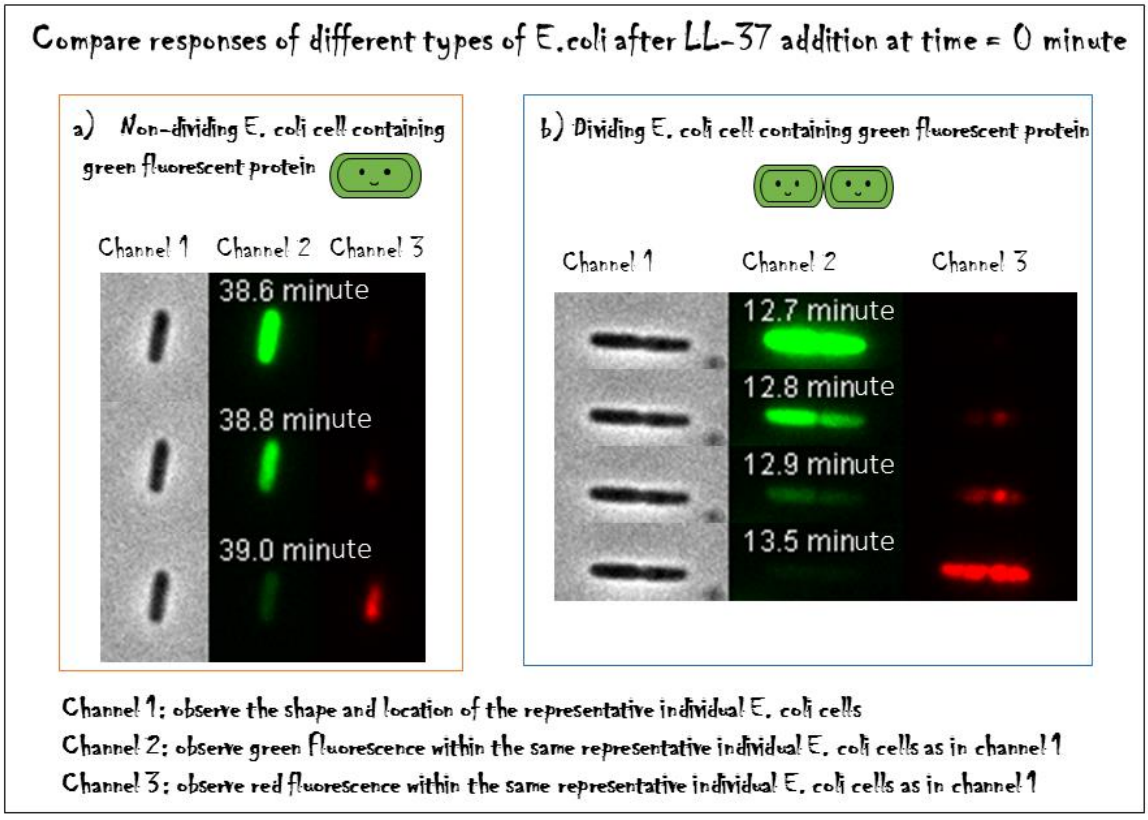


Channel 1 (observe fluorescence within individual E. coli cells)

Channel 2 (observe the shape and location of individual E. coli cells)

Q6: What is the advantage of your technique?

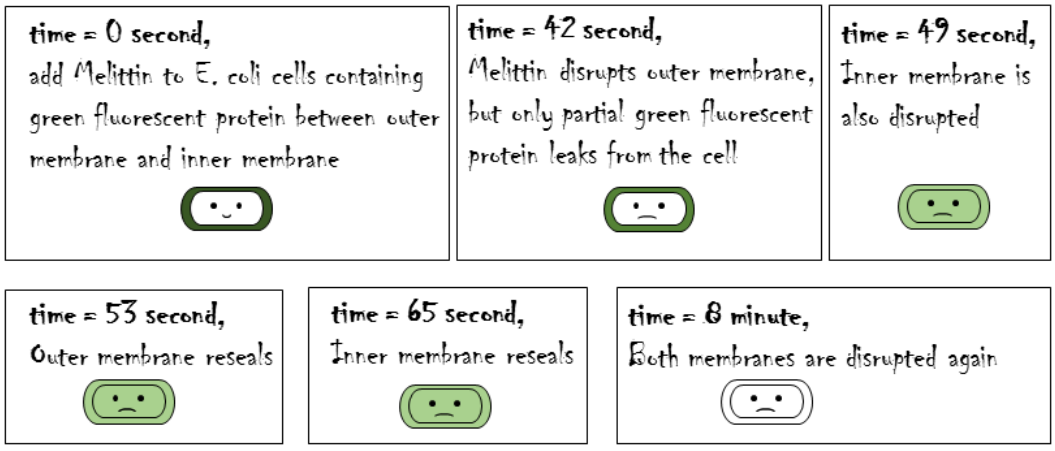
No two leaves are alike, and similarly no two *E. coli* cells are alike even though they have identical DNA. Historically, scientists studied the actions of antimicrobial peptides in an entire population of bacterial cells and assumed that all the cells respond the same. However, the truth might be different. For example, our lab noticed that dividing *E. coli* cells are more susceptible to antimicrobial peptide LL-37. As shown in the graphic below, dividing *E. coli* cells tend to lose green fluorescent protein much earlier than non-dividing cells, indicating that LL-37 broke their membranes much earlier. In addition, the red fluorescence, an indicator for the disruption of the inner membrane, appears at the end of the non-dividing *E. coli* cell first, but first appears in the middle of the dividing *E. coli* cell. This suggests that LL-37 attacks the inner membrane differently on these two types of *E. coli* cells. However, for both types of *E. coli* cells, red fluorescence always appears as green fluorescent protein diminishes. Overall, our technique



provides multiple measurements on the same individual cells and comparisons across different types of cells. It is a powerful technique that reveals a complete picture of antimicrobial peptides' actions on live bacterial cells, giving us feedback for novel antibiotic design and bacterial control.

Q7: What is the major finding in your thesis work?

For the first time, I demonstrated a series of actions of Melittin on both membranes of live *E. coli* cells. In particular, as shown in the graphic below, I figured out both timing and order for outer/inner membrane disruption events, outer/inner membrane re-sealing events and outer/inner membrane re-disruption events. We hypothesize that many antimicrobial peptides disrupt membranes of bacteria, but bacteria then reseal until antimicrobial peptides disrupt again.



In addition, we found that several antimicrobial peptides not only disrupt membranes of *E. coli*, but also induce the formation of harmful compounds inside *E. coli* cells. We also figured out the possible relationship between these two distinct actions of antimicrobial peptides. Overall, my thesis work is enhancing our understanding of antimicrobial peptides' actions in live *E. coli* cells, providing insights into bacteria-related disease treatment and novel antibiotics

development. Many labs in the antimicrobial peptides field showed great interest in our powerful assays. We hope that our single-cell, fluorescent microscope technique can contribute more to the antimicrobial peptide field.

Q8: What is the current stage of developing novel antibiotics from antimicrobial peptides?

As of January 2018, more than ten antimicrobial peptides are in clinical use, such as daptomycin, vancomycin and bacitracin. More are in clinical trials. We hope that more novel therapeutic agents will come into market, saving us from resistant bacteria.