

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 50 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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Understanding Spatiotemporal Aspects of Antimicrobial Peptide Attack on Single, Live Bacteria Using Time-Lapse Fluorescence Microscopy

By

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**Communicating Research to Non-Science
Audience as a Part of the Wisconsin Initiative
for Science Literacy Program**

6.1 Preface

I commend The Wisconsin Initiative for Science Literacy (WISL) for taking on the mission to promote literacy in science, mathematics, and technology. I completely agree and hope that this will attract future generations to careers in research, teaching, and public service. I believe that one of the most crucial aspects of scientific research should be to communicate the research to a broader audience. This is vital to create an awareness of scientific methods and to instill hope and excitement for the future in the general public. I am elated to have this opportunity and I hope to do my best to communicate my doctorate work in the most effective way.

It is consistently proven that visual learning is one of the most powerful learning tools. I have employed this tool throughout this chapter to present my work. The chapter relies on various infographics to convey my research. I hope the readers find this approach more concise, effective, and convenient.

6.2 The Problem: Antibiotic Resistance

The infographics below explain the core of the problem that we are trying to address through our research. This problem pertains to almost all of us in daily life. Antibiotic resistance is both urgent and wide scale in nature [1]. It is important for the scientific community to look for viable and innovative solutions. My research is intended to help generate such solutions.

Antibiotics: Solution for Everything?

Q: Have you used one of these most common antibiotics?

A: Chances are yes!!



• 269 million prescriptions in 2015 in USA: enough for 5 out of every 6 people to receive one prescription each year!

Q: Was it required?

A: Not all of it!!

- At least 30% of the prescriptions were deemed unnecessary
- 9 out of 10 times a sore throat is caused by a virus
- 100,000 tons annual production of antibiotics worldwide!!

Infographic 1: The problem of overuse of antibiotics. The prescriptions numbers were taken from a Centre for Disease Control and Prevention (CDC) report [2]. Antibiotic production number was taken from reference [3].

Antibiotics Overuse: Why Does It Matter?

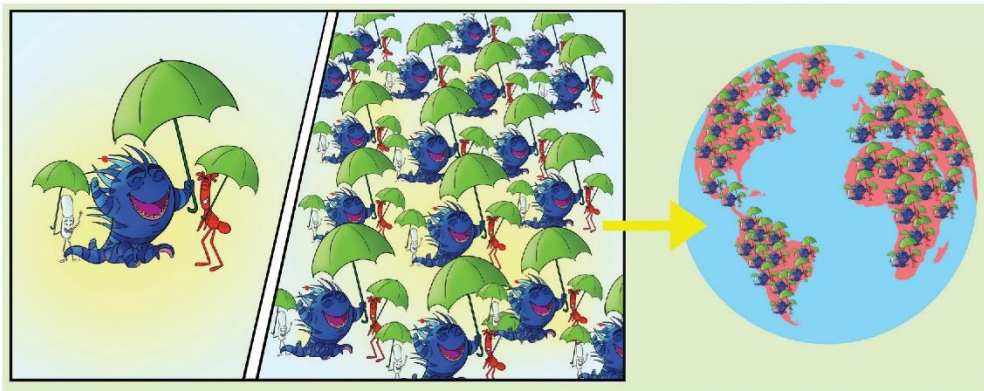
Antibiotics kill most bacteria...



but some can survive and become antibiotic resistant (**superbugs**) and share resistance with other bacteria...



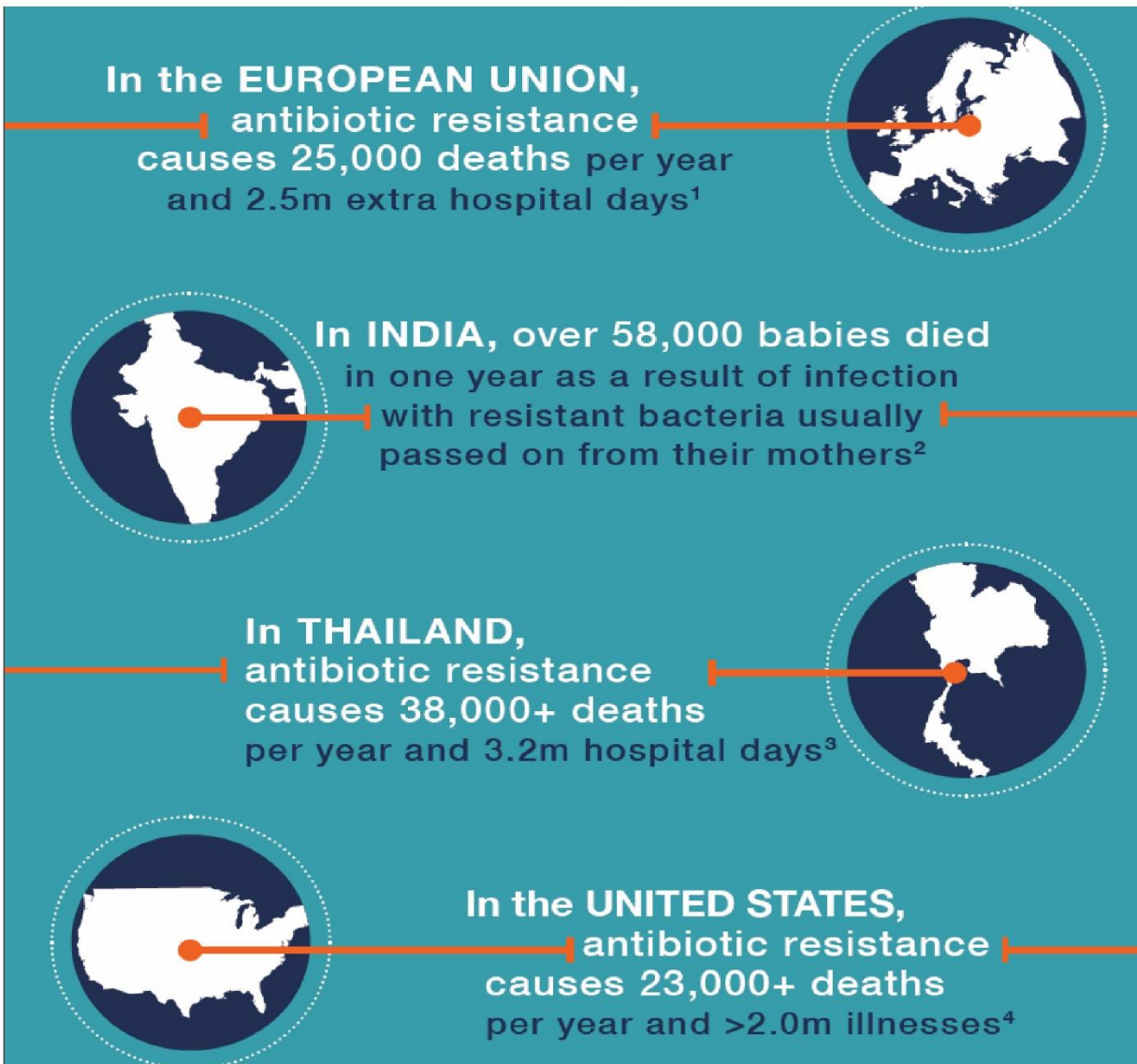
Superbugs multiply and spread everywhere.....



Excessive and unnecessary use of antibiotics accelerate this process of antibiotic resistance

Infographic 2: The emergence and spread of antibiotic resistance [4].

Antibiotics Resistance: Magnitude of The Problem



By 2050, the death toll could be a staggering **one person every three seconds** if antibiotic resistance is not tackled now!



Infographic 3: Antibiotic resistance is a global threat [5].

Antibiotics Resistance: Where Do We Stand?

- Researchers have identified resistance in bacteria against all classes of antibiotics!

XXXX The number inside the box represents the year when resistance against that class of antibiotic was discovered.



No new class of antibiotics has been discovered in the last 25 years!

Infographic 4: Timeline of discovery of antibiotic resistance of major antibiotic classes [6].

6.3 Where can we look for solutions?

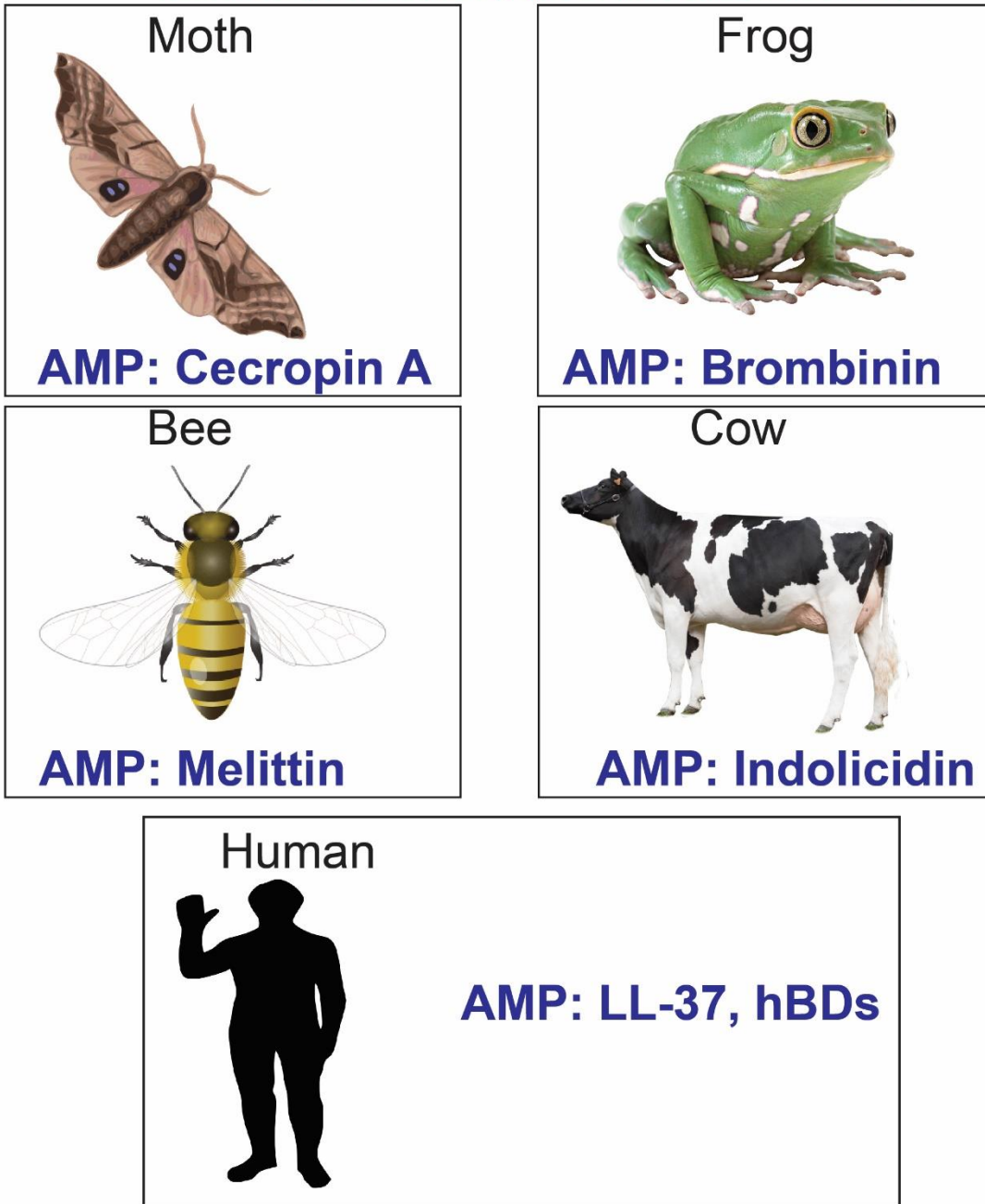
Amidst the growing concern about antibacterial resistance and lack of innovation in conventional antibiotics, researchers have drawn inspiration from the natural immune response of living species. Virtually all organisms produce antimicrobial peptides (AMPs)* to fight bacterial infections [7]. AMPs kill bacteria through a different mechanism compared to conventional antibiotics [7]. A common antibiotic will usually attack a specific target inside bacteria. Bacteria are smart enough to figure out a way to modify the target and hence become resistant to that antibiotic. AMPs kill bacteria in a wide variety of ways. One common one is through disruption of the membrane* of bacteria (see Infographic 6). This promotes the leakage of internal materials of bacteria that are usually essential for bacterial survival. This turns out to be quite a shock for bacteria and it is difficult for bacteria to develop a resistance in these conditions. This makes AMPs an emerging and promising candidate for new antibacterial drugs.

* LEARN A SCIENTIFIC WORD

- **Antimicrobial Peptides (AMPs):** *Antimicrobial:* Anti and microbial, literally translates to opposed to microbial. Microbial is derived from the word microbes. Microbes or microorganisms are tiny living species that can exist in single-cell form or a colony of cells. Cells are the smallest unit of life and are the building blocks of life. Microorganisms include all kinds of bacteria. *Peptide:* Short chain of amino acids, amino acids are essential biomolecules for life that make proteins. ***Antimicrobial Peptides: Chains of amino acids that are harmful to microbes.***
- **Bacterial membrane:** A bacterial cell is surrounded by membrane(s). This membrane structure could have incredible diversity among different kinds of bacteria. The membrane helps enclose the contents of the cell and acts as a protection against outer environment. A common bacteria *E. coli* has 2 membranes called the outer and the inner membrane. The space between the two membranes is filled with proteins and other material essential for bacterial survival (see Infographic 6).

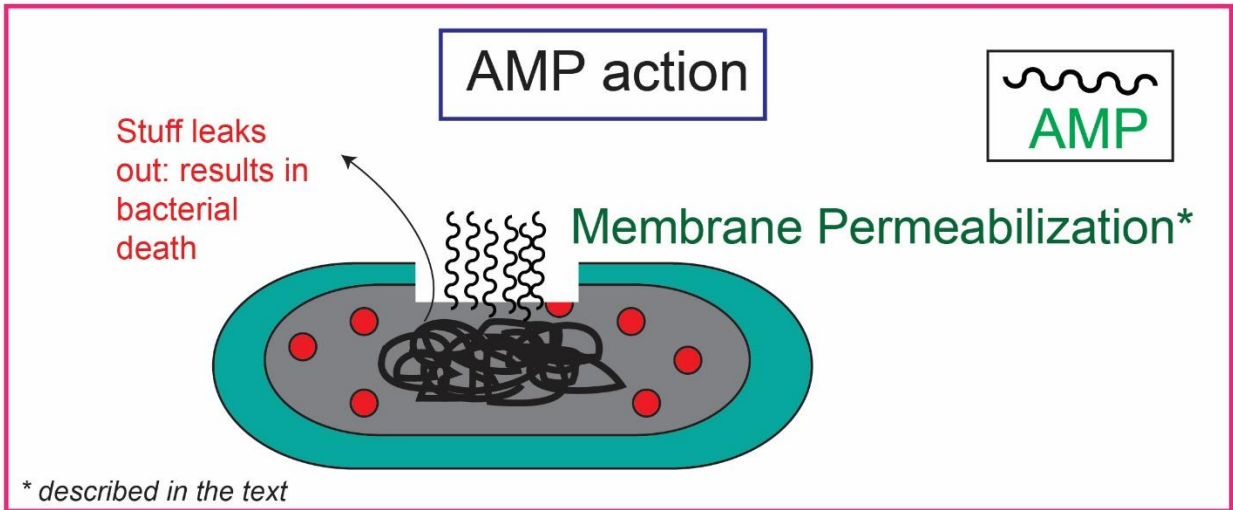
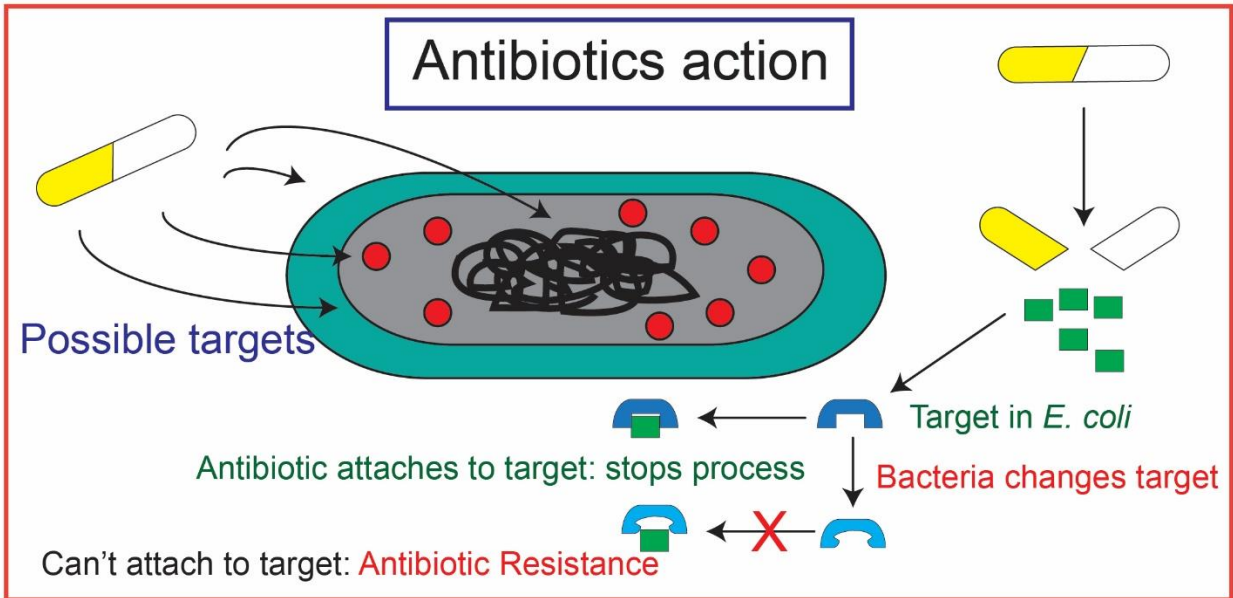
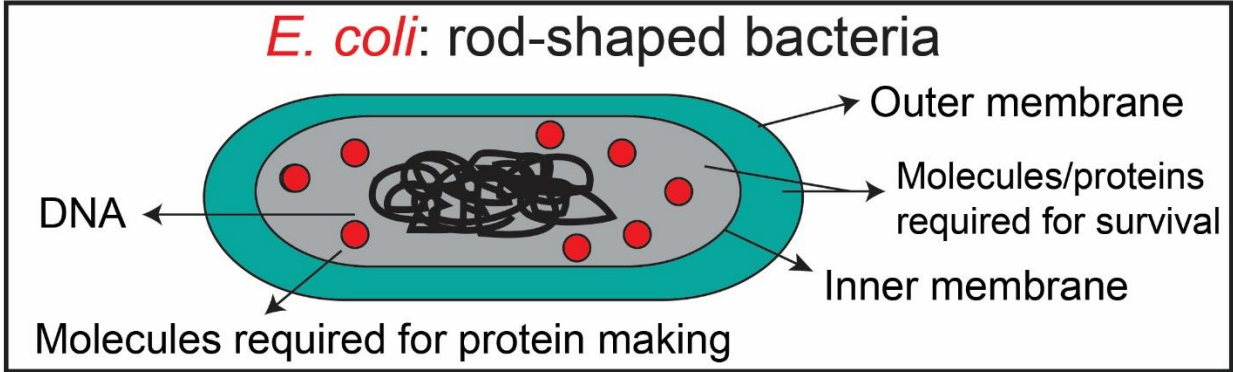
Antimicrobial Peptides (AMPs): Novel Antibacterial Drug Candidates

AMPs: Ubiquitous in living species, first discovered in 1939



***Some AMPs such as CM15 can be made in a lab**

Infographic 5: Major AMPs and their sources [8].



* described in the text

Infographic 6: Schematic representation of a typical *E. coli* cell. General action mechanisms of antibiotics and AMPs are depicted as well.

6.4 Where does my research come in?

There has been increasing interest in AMPs since their discovery in 1939 [8]. There are various efforts focused on discovering new AMPs and understanding their mechanism of action. Our lab is interested in understanding the action of these AMPs on bacteria. We use our expertise to study how these AMPs act on bacterial cells. Our experiments are designed in a way that we can capture images of live, single-cell bacteria in sub-second time resolution. This incredible time and spatial resolution allows us to learn crucial details including when the AMP enters the bacteria, time for membrane permeabilization*, and pattern of membrane permeabilization*.

* LEARN A SCIENTIFIC WORD

- **Membrane permeabilization:** This term refers to puncturing of bacterial membrane. AMPs can cause holes in the bacterial membrane. The holes can cause stuff from inside the bacteria to leak out. This event when the membrane is punctured is called membrane permeabilization.

The infographic below is designed to convey the basic techniques we use to study AMP action on bacteria. In brief, we use a chamber (microfluidics) that has specific channels to maintain a constant flow of AMPs with the appropriate fluorescent dye. First, we flow bacteria in the chamber and observe the cells through a microscope. After that, we flow the appropriate AMP and the fluorescent dye solution in the chamber containing cells. Computer software helps capture snapshots for a duration of typically 30 minutes in constant cycles of time (0.5-6 s). The movie from these snapshots is analyzed and relevant parameters are recorded for each cell. We have used the technique to study various AMPs such as human LL-37, moth derived Cecropin A, bee derived melittin, and synthetic CM15.

How We Study AMP Action: Single, Live Cell Imaging

1



A chamber is used to immobilize bacteria and flow AMP and fluorescent dyes

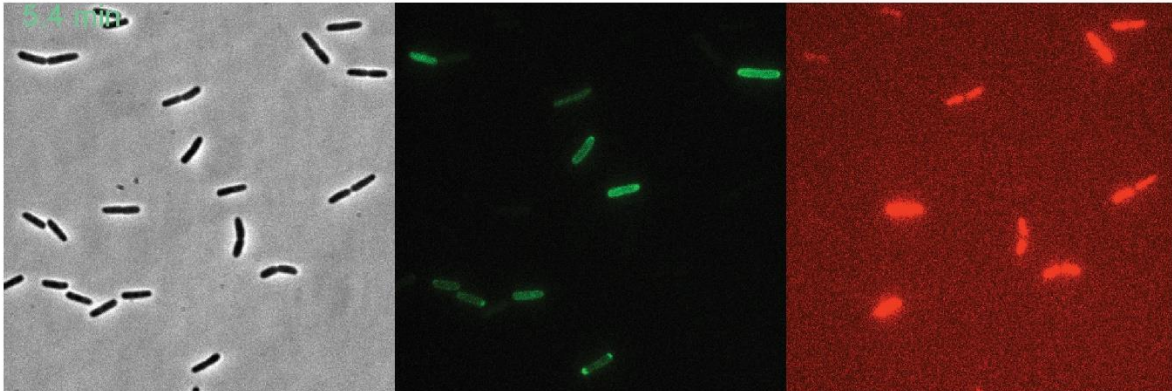
2



Use a microscope to observe the cells

3
Use computer software to get images like this

4



For observing shapes and the length changes of cells

For observing the time and pattern of membrane permeabilization using fluorescence

Infographic 7: Single, live cell imaging experiments used in my thesis work.

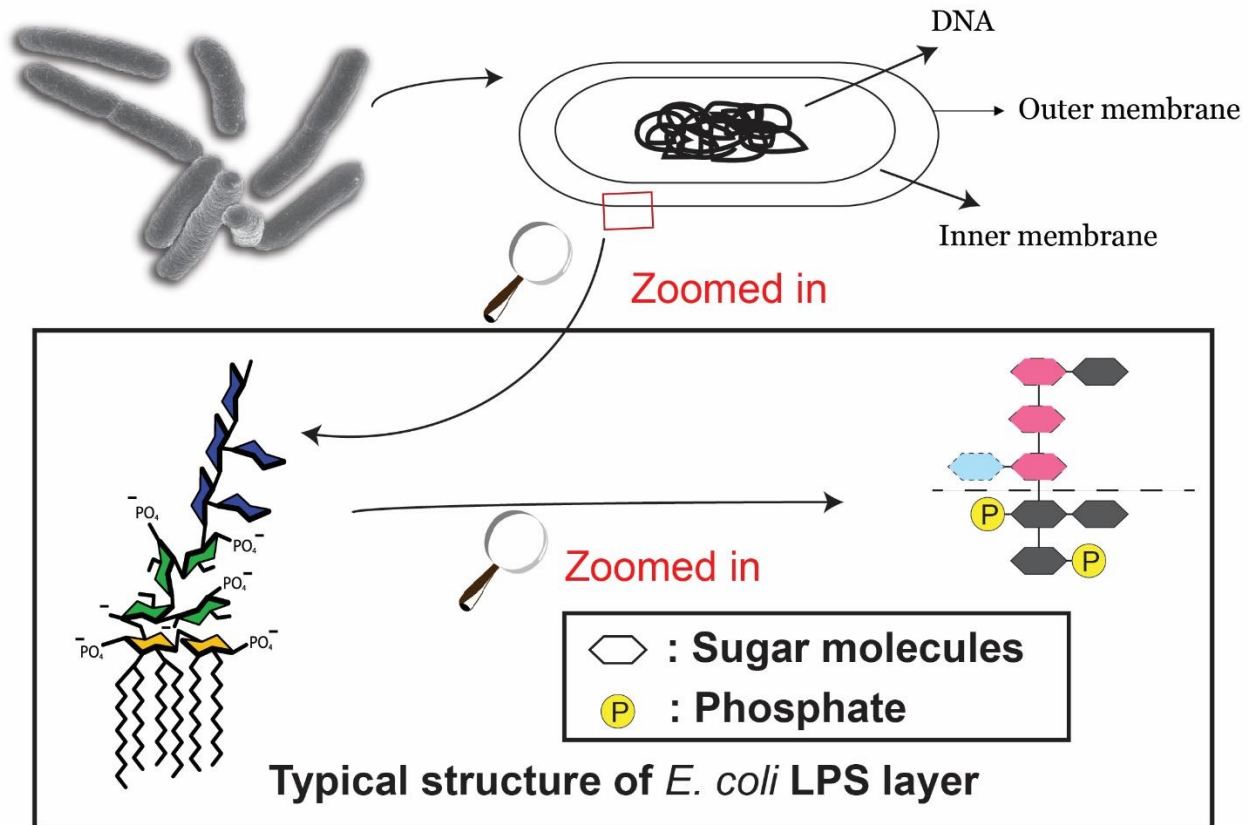
6.5 Current research: Outer membrane layer protects *E. coli* from Cecropin A

As described earlier, we use single, live cell imaging assays to reveal the mechanism for action of AMPs. During my PhD, I extensively worked on a particular AMP called Cecropin A that is derived from moths [9]. In this section, I describe my observations of Cecropin A action on *E. coli*. *E. coli* cells, as described earlier, have two bacterial membranes. The outermost membrane has a layer of sugar and phosphate molecules called the lipopolysaccharide (LPS) layer [10]. We performed experiments with wild-type* and mutant* cells. We observed that the sugar layer and phosphate molecules makes the AMP action slower [10]. This proves that the layer is a barrier for Cecropin A action. We were able to use these observations to propose a model of mechanism for the action of Cecropin A. These observations will help the community learn more about the action mechanism of Cecropin A and possibly assist in designing improved AMPs.

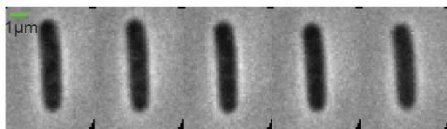
* LEARN A SCIENTIFIC WORD

- **Wild-type (WT):** Most natural form of the particular species. This is the typical form of a species as it occurs in nature. These forms are essential for the experiments to have a baseline for the desired changes.
- **Mutants:** These are the organisms that are generated from a parental, WT strain through mutations. Mutations are achieved through changes in the genetic material (DNA) of a particular organism using biological techniques. Mutations can be natural as well as a result of errors of DNA replication. In laboratory settings, mutants allow observation of behavioral changes resulting from the changes in the particular gene that is mutated.

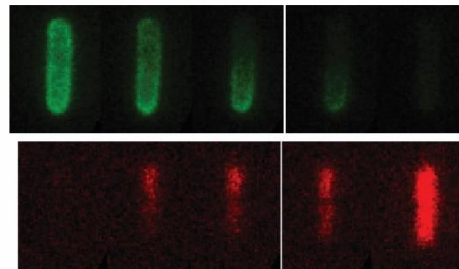
Sugar and Phosphate Slows Down Cecropin A on *E. coli*



Used mutants that have different amount of sugar layer and phosphate



Performed experiments like this



Deletion of sugar layers, or phosphate, or both from the LPS layer

↓ Onset of outer membrane permeabilization

↑ Degree of permeabilization

Infographic 8: Sugar and phosphate protects *E. coli* from an AMP called Cecropin A. The mutants were obtained from the Weibel lab at UW-Madison. The mutants were then modified for our experiments.

6.6 Current research: More Difficult to Kill Food-Starved Bacteria

I extended my work to study bacteria in conditions closer to their natural existence. Typically the experiments are performed on bacteria cells that are in a nutrition rich environment. In these situations, bacteria grow rapidly and also reproduce quickly. However, in nature, bacteria usually exists in very different conditions. There is typically a dearth of food for bacteria in nature to grow very rapidly. In these situations, bacteria change their behavior to a state of minimal growth and reproduction. Since the bacteria change so much in these conditions, it is not accurate to compare it to laboratory conditions. I studied bacteria populations that are nutrient limited. One of the major findings was that more than 10 times the amount of AMP was needed to achieve a comparable membrane permeabilization action to fast growing cells. We were able to identify factors guiding the action of AMP in *E. coli* cells. More studies are needed to understand the behavior and mechanism of AMPs on natural bacterial populations. This will help us design better antibacterial drugs.

6.7 Current research: Improved Design of Synthetic Drugs

Although AMPs are promising candidates for antibacterial drugs, their commercialization has been limited. As of 2018, around 10 AMPs were in clinical use [11]. This limited success can be attributed to several factors. One of the major reasons is the economics of AMP use. AMPs are a defined sequence of amino acids. It is very difficult to synthesize specific sequences and it is very costly. They are difficult to scale and are not very practical. We have collaborated with another lab in UW-Madison to address this problem. In a collaboration with Dr. Leslie Rank and Prof. Samuel H. Gellman at UW-Madison, antibacterial polymers* have been synthesized. These are easy to make and cheaper than natural AMPs. In an effort to help the design of these synthetic drugs, I studied the biological activity of various synthetic polymers. We figured out

certain structural aspects of polymers that help the killing of *E. coli* cells. We hope to use these details to design better synthetic polymers in the future. These polymers could be the new antibacterial drugs that could replace conventional antibiotics.

* LEARN A SCIENTIFIC WORD

- **Polymers:** A polymer is a molecule, made from joining together many small molecules called monomers. The word "polymer" can be broken down into "poly" (meaning "many" in Greek) and "mer" (meaning "unit"). This shows how the chemical composition of a polymer consists of many smaller units (monomers) bonded together into a larger molecule. Polymers can be natural such as DNA, proteins, carbohydrates etc. Some polymers are man-made. Plastics, rubber, and fibers are man-made polymers.

6.7 Conclusions

Overall, in this chapter, I described our efforts to study the mechanism of alternate antibiotic drugs. My research should contribute to the development of new antibacterial drugs. The problem of antimicrobial resistance is urgent and large in scale and serious efforts are needed from the community to combat this resistance. To provide an edge in our fight against bacteria, it is crucial to expand our current knowledge of the mechanism of alternate drugs.

6.8 References

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