Communicating Research to the General Public

The WISL Award for Communicating PhD Research to the Public launched in 2010, and since then over 100 Ph.D. degree recipients have successfully included 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—as well as their excitement for and journey through their area of study—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.

WISL encourages the inclusion of such chapters in all Ph.D. theses everywhere, through the cooperation of PhD candidates, their mentors, and departments. WISL offers awards of $250 for UW-Madison Ph.D. candidates in science and engineering. Candidates from other institutions may participate, but are not eligible for the cash award. WISL strongly encourages other institutions to launch similar programs.

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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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DEFINING PARAMETERS OF SUBSTRATE SELECTION

IN NONRIBOSOMAL PEPTIDE SYNTHETASES

by

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CHAPTER 4: LEARNING FROM BACTERIA HOW TO MAKE NEW AND BETTER MOLECULES

"I have no special talents. I am only passionately curious."

-Albert Einstein

Preface: Science Literacy and Science Normalcy

I live with three non-scientists. Or rather, three curious and creative potential scientists who have not yet undergone scientific training. My spouse is a brilliant scholar of Classics who can tell you the etymology of nearly any word imaginable. My daughters, who at the time of this thesis preparation are 4 and 1, are intrigued by the natural world and regularly make keen observations and ask interesting questions. However, although they are bright, they will likely understand almost nothing reported in this thesis. This is true for nearly all my friends and family members. This is not because these individuals are not smart or gifted. It is because they are not fluent in the language of science - they lack a level of science literacy. However, the findings presented in this thesis, as do all scientific findings, impact all people, regardless of their level of science literacy.

When you walk into a room where everyone is speaking a language that you don't understand, it can feel isolating. If you really want to understand what they are saying, or need to understand what they are saying, it can feel more than just isolating - it can be deeply frustrating. This example scenario, in my view, portrays the current state of science literacy in the general population. Scientists regularly make exciting, ground-breaking discoveries, but typically only write about them in a language that other scientists understand. Part of this problem is lack of training in science communication. Describing complex scientific discoveries is not just "dumbing it down". On the contrary, it is translating your work from the language of science to the English language, and it is very challenging. I am thrilled to have the opportunity to write a chapter of my thesis for a general audience with support from the Wisconsin Initiative for Science Literacy (WISL), who have provided editing and guidance on this chapter.

Scientists, I believe, have an obligation to make their discoveries accessible to the general public, and in making science accessible to the public, we increase science literacy, bit by bit. There is an idea that grows in popularity around the junior high school years that science is "hard", or that you can be "bad" at science, when in reality, science is just something to be learned, like anything else. Essentially, my hope for society is not only an increase in science literacy, but science normalcy. In the same way that most Americans know a little bit of Spanish, or know a little bit about football, or know a little bit about computers, I believe everyone should

know (at least) a little bit about science. In writing this chapter, I hope to make a small contribution to increasing the science literacy of my friends and family, so that they know just a little bit more about science.

Introduction: Useful molecules made by bacteria

Where do medicines come from? Where do we get pesticides that allow farmers to grow their crops at high yields? Where do we get the fragrances and dyes that are used in cosmetics and beauty supplies? You may be surprised to learn that all these molecules are made naturally by bacteria (Figure 4.1). Bacteria cannot communicate by talking, like you and me. Instead, they communicate by making molecules, which convey a message to those living around them.

I'll give you an example: Imagine you are a bacterium living happily on the surface of an apple peel in a trash heap. You are eating the apple peel, and your lineage is rapidly growing. All of a sudden, a new bacterium lands on your apple peel and wants to set up camp. Being a bacterium, you can't ask the intruder to leave. Instead, you make a molecule that will not hurt you or your descendants but will kill the new bacterium. It is in your best interest to make this molecule, so that your lineage can continue to live happily on the apple peel. This molecule you've made is an antibiotic - "anti" meaning against, and "biotic" meaning life.

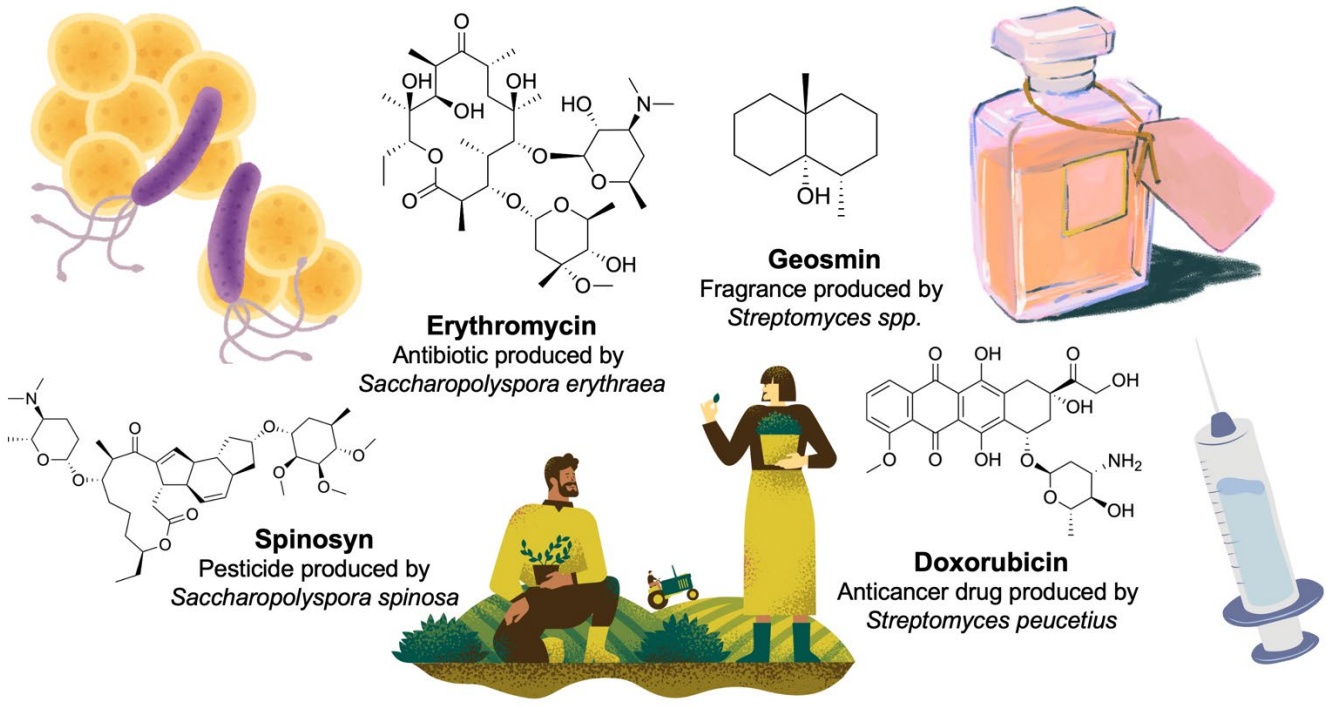

Figure 4.1 Examples of natural products produced by bacteria. Chemical structures for each natural product are shown, as well as the societal role of the natural product and its producing organism.

In this example, the bacterium made the antibiotic to kill off another bacterium that threatened to take over their home. You can imagine, however, that such a molecule would be useful to humans. Indeed, this is the source of many of the antibiotics we use to fight infections in the clinic. This is just one example of how humans use these molecules, which we call natural products. As I hinted at earlier, we use natural products made by bacteria in many different industries, from medicines like cancer drugs and diabetes medications, to pesticides and antifungals for farmers, and many more.

Why we need new natural products

Bacteria (as well as fungi and plants) make hundreds and hundreds of natural products. So why would we need to make new ones? One reason relates to natural products that we use as medicines. A natural product might have some features that make it a great cancer drug, but it might have some undesirable features too, such as high toxicity to the patient, or maybe it has a hard time traveling through the body to get to the spot where the cancer is. For this reason, we would want to make a modified version of the natural product, one that keeps its cancer-fighting ability, while making it safer and more effective for the patient.

Another reason relates to a phenomenon called antibiotic resistance. Antibiotics are critical for a safe and healthy society, but when they are overused in the clinic or in agricultural practices, it can cause a problem. Imagine once more that you are that bacterium on an apple peel in a trash heap. Antibiotics have been overused at a nearby cattle farm, and runoff from this farm has found its way to your trash heap. You and almost all your lineage are killed by the antibiotic. However, one member of your lineage has a random mutation, a change in their DNA that happened before they were born, that just so happens to make them resistant to the antibiotic. Therefore, they can continue to live happily on the apple peel, and they spawn a new lineage, all of which are also resistant to the antibiotic. This phenomenon, where a random mutation occurs and leads to a change that is passed down to new generations, is called evolution. This process typically takes a very long time, but with bacteria we can see evolution happen relatively quickly, because they generally form new generations every few hours, instead of every 20-30 years like we humans do. The more societies overuse antibiotics, the faster bacteria evolve resistance to antibiotics. Therefore, we always need a new supply of antibiotics to protect our societies from infection.

Teaching a new dog old tricks: how can humans make new natural products?

Bacteria have been here for a very long time - so long that it is hard to wrap your mind around. Bacteria were the first life forms on Earth, and fossil evidence indicates they first appeared 3.5 billion years ago. This is 3.1 billion years before the first plants, 3.2 billion years before the first dinosaurs, and 3.49988 billion years before *Homo sapiens* (humans like us)

appeared. They have been making natural products for a very, very long time, and they have gotten very good at it. Bacteria make natural products using special kinds of proteins called enzymes. Enzymes are inside the bacteria, and they allow production of molecules like natural products to occur quickly and efficiently. Humans do not have enzymes in their bodies that can make natural products, so we rely on bacteria to make them for us. Another way to make new molecules is to make them synthetically, meaning by mixing chemicals together in a laboratory. However, some molecules, including many natural products used in medicine, are very hard or even impossible to make synthetically in the lab. The only way to make them is with enzymes. This is why it is important to study the enzymes that make natural products, so that we can learn from bacteria how to make new ones. After all, they've been making them for much, much longer than we have.

What is the first step to making new natural products? To begin, we must gain a better understanding of the enzymes responsible for making natural products. Enzymes grab pieces of molecules from within the bacterial cell and piece them together, like a jigsaw puzzle, until the complete natural product is formed (Figure 4.2). But how do the enzymes know which pieces to grab? This question is the essence of what my thesis has focused on.

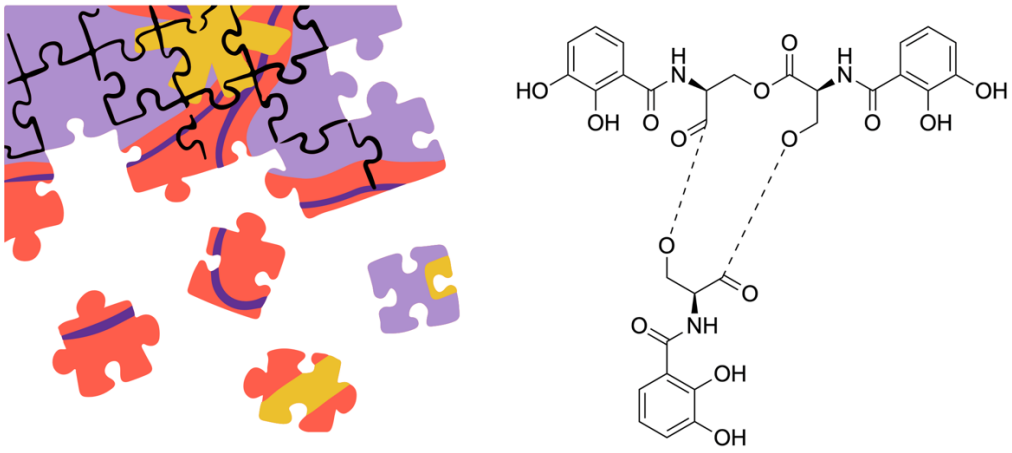

Figure 4.2 Piecing together a natural product. Pieces of molecules from within the bacterial cell are put together by an enzyme to form a natural product, like pieces of a puzzle. Here, the natural product being formed is enterobactin, the molecule made by the enzyme that my graduate research has focused on.

Piecing together enzyme function

Imagine you have a car, and you want to make it faster. However, you have never worked on a car, and you don't know exactly where to start. So, you try changing one thing at a time, to see what effect it has on the speed of the car. You might start by upgrading your spark plugs, then move to optimizing your exhaust system, and each time you change something, you take the car for a spin around town to test your idea. This practice of changing one thing at a time and checking to see what effect it has is exactly how scientists learn things about the world around us, including how enzymes work.

One way that scientists learn how enzymes work is to make mutations. You may remember from biology class that DNA contains the blueprints for how to make everything in our cells, and this includes enzymes. Scientists take DNA out of the bacterial cells, make a change to the DNA that encodes for the enzyme, and then put the DNA back in the bacterial cells to see what effect this change has on the way the enzyme works. This approach, which is called mutagenesis, is the foundation of both of my primary research projects.

Above I described an enzyme grabbing pieces of molecules and putting them together like puzzle pieces. In my first research project, which is presented in Chapter 2 of this thesis, I was interested in learning how to get an enzyme to grab a different molecular puzzle piece than it typically grabs. The word for these molecular puzzle pieces is "substrate" - an enzyme grabs substrates and links them together to form a molecule. This is an important question because if we can learn how to get an enzyme to grab a new substrate, we can get them to make new molecules, such as less-toxic medications or new antibiotics. To do this, I mutated the DNA that makes the enzyme in many different ways to make millions of enzyme variants. For this study I only mutated within the exact space that the substrate touches the enzyme, which is called the "binding pocket" (Figure 4.3). Then, I tested all the enzyme variants to see if the mutations caused them to function differently. Specifically, I tested to see if the enzymes could make a molecule that binds iron. The only way they could make a molecule that binds iron is if their mutations caused them to grab a new substrate. In this study, I did find some enzymes that grab a new substrate, and to our surprise, they made a brand-new iron binding molecule that has never been reported before. I then looked at the DNA to see what mutations caused the enzyme to grab a new substrate. Excitingly, when I put the same mutations into two other enzymes, they grabbed the same new substrate. This confirmed that I had figured out how to get enzymes to grab this new substrate.

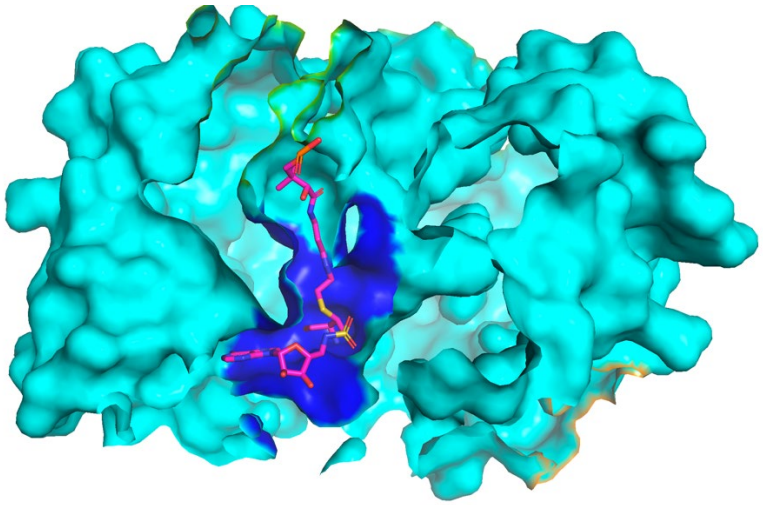

Figure 4.3 Model of the binding pocket of the enzyme EntF. EntF is the name of the enzyme that my graduate research has focused on. The model shows part of the enzyme, the part that grabs the substrate, sliced in half to reveal the binding pocket, which is shown in dark blue. The substrate is shown in magenta.

My second research project, which is presented in Chapter 3, also involves making changes to enzymes, but to answer a different question. For this study, I wanted to figure out what parts of the enzyme are absolutely essential for function. This is important because researchers have been trying to get enzymes to grab new substrates for a long time, but most of these attempts have not been successful. This is because we just don't know what parts of the enzyme to change, and what parts need to be left alone. To answer this question, I again mutated the DNA that makes the enzyme, but this time I mutated a much larger part of the enzyme, not just the part that touches the substrate. Some scientists have found that changes at parts of the enzyme that are not even close to the substrate can have surprising effects, so I wanted to see if this might be the case for the enzyme I was studying. In this study, I did a very thorough mutagenesis. I actually made every single possible change you could make to the enzyme, and then I tested them to see what effect the changes had on enzyme function. In this experiment, I was not trying to get the enzyme to grab a new substrate, like in the other project. Here, I was just trying to see what effect the changes had on the enzyme grabbing its normal substrate. This study identified parts of the enzyme that were critical for function, some that had not been shown to be important for function, and some that were not even close to the substrate. These results expand what we know about how these types of enzymes function, and highlight the importance of analyzing parts of the enzyme beyond the binding pocket to understand substrate selection.

Together, the findings from my graduate research help scientists make new natural products, by informing them what parts of the enzyme that make these molecules are important for function, and even how to get an enzyme to grab a new substrate to make a new molecule. The work I've done is in one model system, and additional work is needed in different enzymes and different model systems to get a complete understanding of how enzymes grab the right substrate and how to change which substrate they grab. Studying these enzymes and learning from bacteria how to make new molecules is exciting work, and I am privileged to have had the chance to contribute to our understanding of these enzymes.