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.

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FRET-based biophysical characterization of bacterial divisome transmembrane proteins

By

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APPENDIX II

The FRETting tendency of bacterial proteins

I feel fortunate to be able to contribute my scientific work to the broader society through this platform of expression. I would like to thank the efforts of the Wisconsin Initiative for Science Literacy (WISL) and the AAAS-sponsored Dance your PhD contest for providing us the microphone to reach the wider public.

Note: Please visit http://www.news.wisc.edu/22449 for a video containing a depiction of the following work in the form of an interpretive dance. The video provides a visual to go along with the methodologies used in my work, which I have described in this chapter.

Introduction

What is the problem to be targeted?

Most of the research in biological sciences these days tackles a problem. And for us, humans, a biological problem means a disease. The word disease can be broken into dis–ease, which means something within us is not at ease. There is a reason for the state of dis-ease, and our job as scientists, is to research, or find the root cause of that dis-eased state, and try to rectify it. We are aware of the various kinds of dis-eases that bother us in our lives – the smaller kind such as cold and flu, and the ones that don't have a cure, such as cancer. The reason cold and flu aren't life threatening anymore is because the root causes of those dis-eased states have been identified and tackled. For cancer, the search continues...

One example of a dis-ease is a bacterial infection. Bacteria are micro-organisms that are present everywhere – in our houses, in our food and even inside us. Based on the type of bacteria, there are those that are harmful to us, and those that we can tolerate. Some bacteria reside in our gut, some are present in yogurt and so on. But there are others that cause problems or 'infections' such as the one that causes the commonly occurring 'strep throat'. In the case of an infection, bacteria enter our body through some form (food, air, contact with another living organism), decide to mark their territory in one part of the body, and start feeding on us and multiplying. One bacterium takes the nutrients it needs from our body, grows in size, and then splits into two new bacteria. The two new bacteria will then take up more nutrients, grow in size, split into two more to make four bacteria. This way there will be more and more bacteria in our system, feeding on us, and making us sick. One rod-shaped bacterium is 1 micrometer in size, so you can imagine, that in a 1 cm x 1cm area on your body, there can be 10,000 x 10,000 = 10^8 bacteria at work!

Our body has a natural defense mechanism which usually works very well to fight off and kill these unwanted organisms. But each individual is made differently, and based on the strength of the individual's fighting machinery or 'immune system', they can either ward off a bacterial invasion, or get 'infected'. In such cases, doctors prescribe us 'antibiotics'. These medications are designed such that they will attack only the bacteria and not us humans. So when we consume these antibiotics, they target the bacteria, prevent them from carrying out the processes they need, to grow and survive, and the bacteria eventually die. Infection cured. There is a catch, however. These bacteria are smart, and once they get an idea of how the drug is designed to attack them, they slowly modify themselves such that the drug doesn't work on them anymore! And we get what we know today as a superbug! Bacteria that are 'antibiotic resistant'. For bacteria to modify themselves, all they need is familiarity with the drug. The more frequently we use antibiotics, the more familiar they become, and the more resistance these bacteria develop to them. *The CDC (Center for Disease Control and Prevention) generated a threat report in 2013, showcasing an alarming number of over two million illnesses and about twenty three thousand deaths caused by infections due to antibiotic resistant bacteria in the United States alone*. So how do we tackle this serious problem?

How do we target this problem?

One way is to prevent overuse of antibiotics. This is difficult because a lot of antibiotics in the market are broad-spectrum, which means they can treat different kinds of bacteria. When we take in an antibiotic, it may kill a certain type of bacteria, but at the same time present itself to another type of bacteria for familiarization, and make that bacteria modify itself and be resistant to it. Next time if the same antibiotic is used on an infection by this

modified bacteria, it may no longer be effective, and the infection would take over. Antibiotics work by entering the inside of the bacterium, and affecting some of its very basic cellular processes; which it requires to fully function and live. ***Before going into further detail, let us zoom in a bit to understand this better.***

As we have all heard, the most basic unit of life is a cell. A cell is what carries out all the process required for life. Multiple cells of a certain type club together to form different organs in our body. Our lungs are made of cells that help us breathe, our heart is made of cells that can help circulate blood, blood itself is made up of different kinds of cells like red blood cells, white blood cells, and so on. Zooming in further, we are now looking at the inside of the cell. Imagine a factory, with thousands of processes simultaneously going on. There are millions of people carrying out their jobs in a concerted fashion. Some are carrying cargo from one end of the factory to another, some are building the architecture of the factory, some are welcoming newer people into the factory who have just arrived after being fully trained to work, some are managing poor workers and eliminating them. Each of these processes are necessary for the proper functioning of the factory, and the concerted effort of all these people is what makes that possible. These 'people', inside a cell are known as 'proteins'. Proteins are of various kinds and carry out various cellular processes. Some proteins are involved in the synthesis of other proteins as well as synthesis of protein precursors. Without these essential proteins, the cell will not function, and eventually die.

The antibiotics for bacterial infections in the market work by attacking some proteins inside the cell that are required for synthesis of other proteins and protein precursors. The reason such antibiotics are available is because these processes of protein synthesis etc. are very well understood, and thus drugs to alter these processes have been designed. But as

described earlier, the problem of antibiotic resistance is growing.

Now, unlike humans that are made up of millions of cells, a bacterium is made of just one cell. The cell itself is the bacterium. As I mentioned earlier, it carries out all its cellular processes, grows, and then splits into two new cells. The process of splitting into two new cells is called cell-division. Some proteins carry out the cellular processes needed for the cell to function and grow, and some proteins are needed for cell-division. These proteins required for cell division of the bacteria are not really present inside the cell, but are part of an outer layer of the cell called the cell-membrane that separates a cell from its outside environment. **Thus, these proteins are called membrane-proteins**. When the cell is ready to divide, these membrane proteins all come together in the middle region of the cell membrane, interact with each other, and start deforming the cell there so that it can prepare to split in two.

Designing drugs that target the membrane proteins that carry out cell division, can be another approach to tackle bacterial infection. But the reason this approach is not fully used, is because the process of cell division is still not very well understood. The different kinds of membrane proteins involved in the process are known, and we also know that these membrane proteins come together in the cell membrane and interact in some way to enable cell division. It has also been studied and shown previously that in the absence of membrane proteins, cell division cannot occur. But exactly which proteins are partnering with each other and forming what sort of a structure, a set-up that is important to know in order to design drugs, is still not well understood. Further, we don't know exactly how these proteins communicate with each other as well.

Thus, the focus of my work is to better understand the process of cell division in bacteria by studying the membrane protein interactions, in an effort to target the problem of antibiotic resistance.

The specific question

To begin understanding the complex interactions of the membrane proteins involved in bacterial cell division, we started looking at the interactions between two such proteins, FtsB and FtsL. We know from previous research that FtsB and FtsL do form a complex, meaning, they are interaction partners. It has also been shown by other research groups that the two proteins are essential for each other's viability in the cell membrane, and that upon interaction, they can recruit the remaining proteins needed for completion of the cell division process. But exactly how they interact with each other, that is, which parts of the two proteins are involved in interaction, is not known.

Now, if we take a look at the architecture of these two proteins, both have a component that is embedded in the cell membrane, and a component that is present right outside the cell membrane (called the periplasmic region). In order to start mapping the interaction regions, we decided to divide them into individual components (membrane region and periplasmic region), and investigate which regions are required for the two proteins to interact. For my PhD, I have focused on the membrane regions of FtsB and FtsL in order to understand the role of the membrane region of these proteins in the FtsB-FtsL interaction, and thereby in bacterial cell division.

The technique used

To study proteins or, for that matter, any molecular component of a cell, there are three broad approaches scientists use in our field – *in vivo, in vitro* and *in silico*. *In vivo* refers to studying the molecules in their native environment, that is, inside a cell or inside an organism,

such as the studies performed on mice. In this approach we employ techniques like microscopy and fluorescence methods where the molecules are genetically modified to be tagged or labeled with fluorescent molecules so that their behavior in their native environment can be monitored from the outside. The advantages are that we are observing the molecules in their natural state along with their natural components, but the disadvantages are that we cannot alter the environment too much to observe its effects on the molecule in question. *In vitro* on the other hand refers to taking the molecule in isolation and studying it. Here the disadvantage is that we don't look at the molecule in its native state, but we have more control over the modifications on the molecule and the environment, and can obtain more physical parameters. A third approach is *in silico*, or computational analysis. *In silico* analyses observe the molecule's parameters computationally, and can perform complicated modifications to the molecule in much lesser time to observe its theoretical effect. Computational modeling of biomolecules has been a tremendous breakthrough in aiding and complementing experimental research in the biological sciences. A comprehensive analysis of any biological system employs a concerted effort of all three approaches.

In my work, I have focused on studying the interactions between the membrane components of the two cell division proteins FtsB and FtsL using an *in vitro* approach. To do so, I first chemically synthesized the two proteins using a chemical synthesis technique. In this technique, the building blocks of the proteins are added stepwise in a certain sequence that is characteristic to the identity of the protein. Each protein is synthesized in this manner until they mimic the natural composition of their membrane component. Once the synthesis is completed, the proteins are chemically labeled, or tagged with fluorophores (molecules that are fluorescent upon shining with light) at their ends. Since fluorescent molecules have a specific color, or a specific behavior under light, tagging the proteins with fluorophores allows

me to visualize their behavior. Once the labeling is achieved, the reaction is subject to a purification procedure that separates the labeled proteins from the unlabeled proteins, allowing me to perform the experiments with pure labeled proteins. Finally, using the pure labeled FtsB and FtsL proteins, I carry out the experiment that enables me to look at the interactions between these proteins, and to do this experiment, **I use a method known as – FRET**.

FRET stands for Förster Resonance Energy Transfer. In this technique, one fluorophore transfers its energy to another fluorophore if the two are in close proximity to each other. These two fluorophores are called a FRET pair, and need to be chosen based on their behavior under light. For example, if we look at the spectrum of visible light going from low to high wavelengths, it actually is split between different lights in the form of VIBGYOR, or Violet-Indigo-Blue-Green-Yellow-Orange-Red. So when a blue fluorophore is excited by shining light of an adjacent lower wavelength on it, such as Indigo,, it will exhibit blue fluorescence. Similarly when a green fluorophore is excited by shining blue light on it, it will exhibit green fluorescence. This means that for the green fluorophore to be excited, it requires light energy corresponding to blue light. Now, if the blue and green fluorophores are in close proximity to each other, then if we shine indigo light on them, the blue fluorophore will get excited; but instead of exhibiting its blue fluorescence, it will transfer that energy to the green fluorophore which is of the right energy to excite it, and we will observe green fluorescence instead. This phenomenon of energy transfer between two fluorophores in close proximity is called FRET. Thus, FRET can be used to study interactions between two proteins. If the two proteins are attached to, or labeled with fluorophores that form a FRET pair, then we will observe FRET in the form of green fluorescence if there are interactions that exist between the proteins.

FtsB and FtsL are membrane proteins, and in my work I used FRET to study the

interactions between their membrane components. This means that to understand the natural interaction tendencies of these two proteins, I needed to study them in an environment that is close to the composition of the cell membrane. Now, the cell membrane's composition is very diverse – it is made up of different kinds of molecules comprising various sizes and properties, but the most important characteristic of the membrane is that it is 'hydrophobic', or water repelling. It is made of molecules that are lipidic, or 'greasy'. Thus, the proteins that reside in this part of the cell are also greasy in composition and do not dissolve in water. This makes studying membrane proteins complicated, because unlike proteins that are present inside the cell (which has a high percentage of water), membrane proteins cannot be studied in water-based solvents *in vitro*. Therefore, to study membrane proteins using FRET or any other technique *in vitro,* we first need to dissolve them in artificial lipid environments like detergent based solutions, or small artificial membranes that try and mimic the natural membrane environment. This provides a habitat close to their natural state, and allows us to study the proteins with the assumption that their behavior in the natural cell membrane is similar to the artificial lipid membranes we synthesize.

So, once I synthesized, labeled and purified the FtsB and FtsL membrane proteins, I added them to artificial membranes and performed my FRET experiment. In one reaction, I had FtsB labeled with blue fluorophores only. I excited them with indigo light, and observed blue fluorescence. This told me that the fluorophores are behaving well in the artificial set up. In another reaction, I took FtsL labeled with green fluorophores only. Again I excited them with indigo light, and observed nothing. This was as expected, because indigo light doesn't have the exact energy/wavelength to excite a green fluorophore. In a third reaction, I mixed the two proteins – blue labeled FtsB and green labeled FtsL. Now when I excited this reaction with indigo light, I expected to observe one of two things – either blue fluorescence only, indicating

that I am exciting the blue FtsB proteins and they are not transferring their energy to green, or lesser blue fluorescence compared to my first reaction, and an additional green fluorescence. The second situation would arise if a green fluorophore will be in proximity to the blue fluorophore, indicating that FtsB and FtsL are close to each other, and thus interacting. I performed these experiments in very dilute conditions such that any FRET observed was only due to specific interactions between FtsB and FtsL proteins and not just due to a crowded environment with proteins 'bumping into each other'. The extent of the interaction could be estimated by the amount of decrease in blue fluorescence observed, compared to the first reaction with blue-FtsB alone. With this experiment, I found that FtsB and FtsL did exhibit the second situation of FRET with appearance of green light and decrease of blue light indicating that they have a very high tendency to interact through their membrane components alone.

Conclusions

In our lab, we study interactions between membrane proteins using various techniques. For my dissertation, I focused on studying the membrane components of two proteins FtsB and FtsL that are required for cell-division in bacteria. Using a technique known as FRET, I observed that the two proteins contain a membrane component that drives their interaction to form a FtsB-FtsL complex. In addition, I also observed that FtsB alone has a tendency to form a 'dimer', or a pair with another molecule of itself. This led us to believe that there must exist some sort of competition between one FtsB molecule and one FtsL molecule to pair up with the other FtsB molecule. On performing FRET studies on the different combinations of these proteins, I found that these two proteins probably interact in a manner where the interaction complex is not just made of two proteins but contain a higher number of each of the proteins. I found that the number of FtsB and FtsL molecules in that complex has

to be equal, and thus we think that the way these two proteins interact is that FtsB alone forms a complex with two or more molecules of itself, and then two or more FtsL molecules 'dock' on the FtsB complex to form a higher complex.

Whether this complex is made of two molecules of each protein or a higher number of molecules of each protein, is still unknown. But what we gather from this piece of information is that during the process of cell division in bacteria, these two proteins form a big interacting complex. We also know from previous research that other proteins needed for cell-division interact separately with FtsB and FtsL. Thus we think that by forming a big FtsB-FtsL complex, these two proteins bring other cell-division proteins together in the membrane and facilitate cell division.

Membrane proteins are hard to study because maintaining them in their native state and designing artificial cell membrane-like media to keep them 'happy and healthy' is quite a challenge. Information on the FtsB-FtsL complex is only one piece of the puzzle as to how cell-division in bacteria works, but it is the first piece of information on the interaction characteristics of the membrane components of these proteins, and is a start! As a research group, we continue to work on the different components of all these proteins separately. Using various techniques and approaches, we aim to slowly put the pieces of the puzzle together toward the bigger goal of understanding how bacteria divide and multiply, so that they can be manipulated in order to tackle the ever growing medical problems of bacterial infections and antibiotic resistance.