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.

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.

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Dynamics of EF-Tu/ternary complex in live *E. coli* using superresolution imaging

By
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Chapter 6:

Why is protein production so efficient?

Communicating research to a non-scientific audience as a part of the Wisconsin Initiative for Science Literacy (WISL) program.

I greatly appreciate the initiative taken by WISL, which allows me and many others to explain our research to a non-scientific audience. All scientific research is done with the goal of being useful to many people. Thus, it is imperative that we get to communicate our findings with others. Thanks to all the members of WISL for providing this platform.

6.1 What kind of chemistry do I do?

Technically, I am a physical chemist, but my research is mostly biological. Thus, I am, more appropriately, a Biophysicist! In physical chemistry, we try to understand how different materials or substances interact with one another. Materials can interact in many ways – similar particles can interact among themselves, or two different particles can interact and lead to the formation of a new substance, etc. Physical chemists are mostly interested in how fast or strong the interactions are, or what kind of energy change is involved, or some other observables like how many particles are interacting. However, as I stated above, I study mostly biological substances, specifically the interactions of different proteins – the entire dynamics of their interaction. This might still seem vague but hopefully should be clearer in the following paragraphs.

6.2 What is my research about?

Our lab – The Weisshaar lab, is dedicated to learning how different biological systems function inside any organism. The smallest block in every organism where complex biological processes occur is a cell. Each cell consists of DNA, RNA and many different proteins. Some examples of these processes are - making different proteins, uptake of nutrients, changing the form of the nutrients, making another copy of DNA, etc. Different cellular systems or machinery carry out different processes and each system includes multiple proteins working together, each

with a different function. The number of cells in an organism vary – Humans have billions of cells in our body, but bacteria only has a single cell. Many of these processes in different organisms share quite a lot of similarities. Thus, we chose our model organism to be a specific bacterium called *E. coli*, as this is a relatively simple system to study. There are different ways to approach the question of understanding cellular processes and the most obvious or the most popular approach is to lyse the cell or disintegrate the cellular membrane by physical or chemical means, which spills all the cellular content. This method allows us to study only that specific protein system. This approach is called *in vitro* study. This approach can also be described as studying a specific cellular system outside the cellular environment. *In vitro* experiments give us a lot of control over what we are studying and has helped the whole scientific community learn a lot about these systems. Scientists have figured out how fast different proteins can interact with one another to carry out their biological functions. Even though it is important to know the rate of such interactions, the results from these experiments can be significantly different from the actual rates. The major issue with such experiments is that the cellular environment can be very different from the *in vitro* environment. This environment plays a major role in determining how fast a protein can move and find its interacting partner. Crowding can slow the motion of a protein but can also bring its interacting partner closer. It has also been observed that the binding interaction between proteins is stronger inside cells compared to *in vitro* [1]. Our lab's research has been focused on studying the live cell dynamics of different cellular processes. My research has been focused on understanding the dynamics of a specific process called Translation inside live cells.

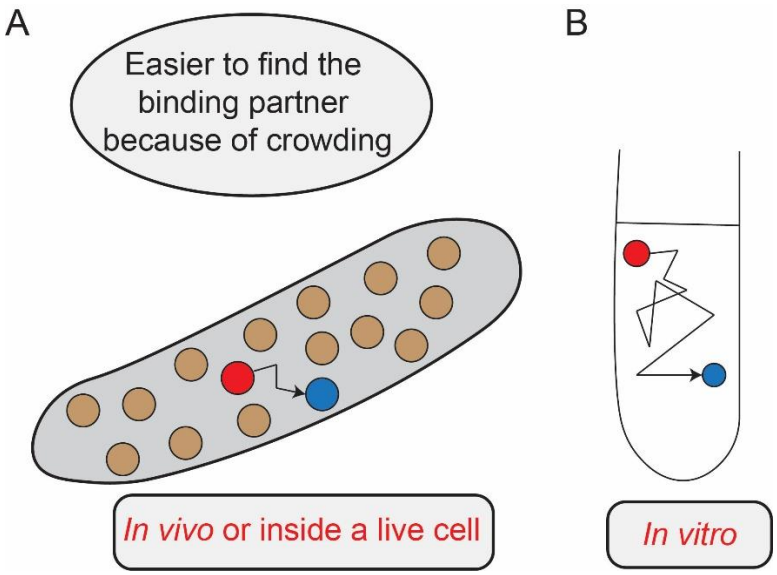

Fig 6.1: A) Cartoon representation of protein interaction inside a bacterial cell. Due to crowding from other proteins, the rate of protein interaction can increase as they can come closer to each other. **B)** Cartoon representation of *in vitro* conditions. In this example the rate of interaction is significantly lower in *in vitro* conditions. Proteins are not drawn to scale.

6.3 What technique do we use?

A very efficient way to visualize what happens inside a live cell is by fluorescence imaging. To understand this technique, we should have some background about fluorescence. There are certain materials that when illuminated by light of a specific color, absorb that light and emit light of a different color. This emission is called fluorescence. Not all materials show fluorescence. The ones that do are called fluorophores. Many materials are fluorophores, including some proteins. For our imaging experiments, we attach a fluorescent protein to the protein we want to study, and now the fluorescent light can show us how the protein of interest is moving around. We look at these fluorescence emissions under a microscope to understand the dynamic behavior of the protein.

This seems very straightforward and an efficient way to study protein dynamics. But this technique has a huge limitation – the resolution. Anything smaller than ~250 nm cannot be seen under a microscope [2]. They appear as a blob of 250 nm radius. To give a perspective of the size, human hair is around the thickness of ~0.1 mm (1 mm = 1,000,000 nm), which is around 400 times larger than this resolution limit. Thus, substances as small as hair can easily be seen in a microscope. Then what is the issue? Well, the average protein size is around 2-10 nm. Thus, it is around 10,000-50,000 times smaller than the thickness of human hair. Also, a bacterium itself is around 4 μm long and 1 μm thick (1 μm = 1,000 nm). Thus, it is 100 times smaller than hair. The proteins inside the bacteria are not very far apart and usually there are thousands of them. So, it is very difficult to tell them apart by fluorescence microscopy.

In 2006, three research groups had a breakthrough in the resolution limit problem of microscopy [3-5]. They had figured out a way, by using special fluorophores or fluorescent proteins, to achieve resolution around 30 nm. Now, there was a way to study these protein interactions inside the crowded bacterial cell. This superresolution imaging technique was called Photoactivatable Localization Microscopy (PALM). Prof Eric Betzig received the Nobel Prize in 2014 for its development. For my research, I use the PALM technique to visualize translation of specific proteins in a cell and understand their dynamics inside the live cell.

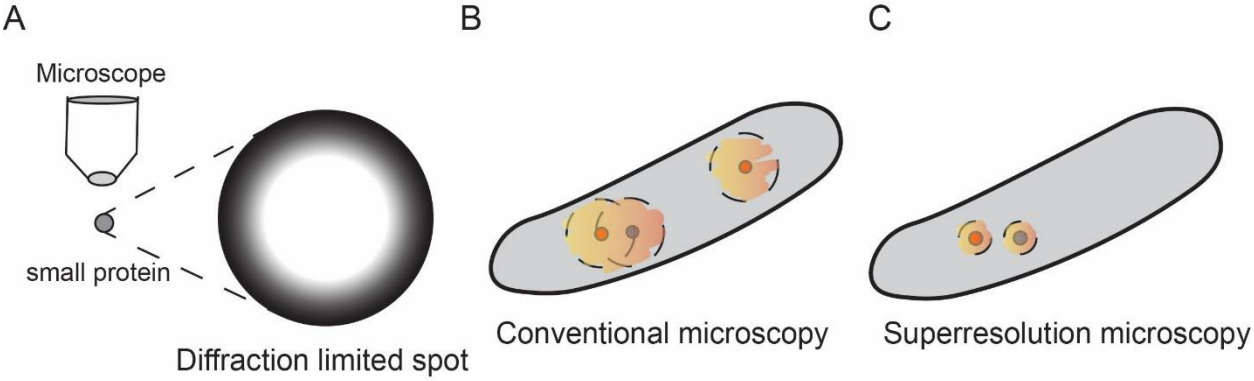

Fig 6.2: A) When the fluorescence of a small protein is viewed under a microscope, it appears as a giant blob of light. This limits our ability to differentiate 2 proteins when they are close to each other. **B)** Figure showing how the fluorescence image of two small proteins present very close to each other inside a bacterial cell will appear. The yellowish orange region represents the fluorescence image. **C)** Figure showing how the fluorescence image of the same proteins look by using superresolution microscopy. Now, it is much easier to track individual proteins.

6.4 What is translation and what have I discovered?

Translation is an essential and complex process in which new proteins are formed in a cell. The process involves numerous different proteins working in tandem. The most important component involved in translation is called a Ribosome. Ribosomes are huge complexes made up of both proteins and RNA. They are the largest component of a bacterial cell. The term DNA might be familiar to most. It is the genetic material inside every organism. Differences in DNA among individuals lead to differences in physical features, etc. There is another material which is like DNA inside every organism called RNA. RNA is produced from DNA by a process called transcription. Similar to DNA, RNA is coded to determine which proteins should be produced and in what quantity. When RNA is formed, they travel to the ribosomes, which then decode the RNA and make proteins. Many proteins help the ribosomes in this process. One such protein is

Elongation Factor-Tu (EF-Tu). EF-Tu binds to a specific region of a ribosome and helps start the protein making process [6].

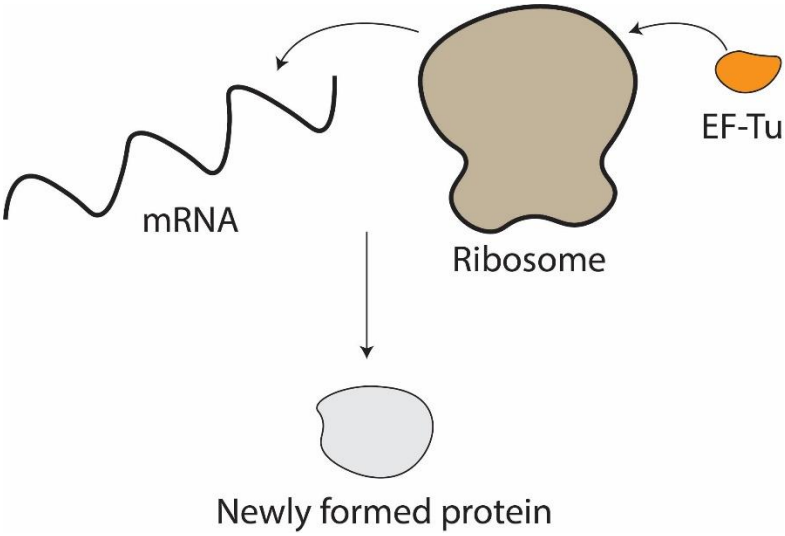

Fig 6.3: Schematic representation of how the translation process works. Ribosome binds to the mRNA (messenger RNA) and EF-Tu binds to the ribosome to start the translation process. The product is a newly formed protein.

I used PALM to study how exactly EF-Tu interacts with ribosomes and found few, previously unknown, interesting components in the Translation pathway. First, I was able to estimate the speed of this interaction, which helps us understand the rate of the entire translation process. My measured rate was drastically different from the rate measured by *in vitro* experiments, which were deemed to be very slow. I also observed that multiple EF-Tu can bind to the same ribosome, which helps us understand why Translation process is so efficient and why the rate of translation is so high [7].

After developing a proper understanding of the EF-Tu dynamics in live cells, I tried to understand if the motion of EF-Tu in the bacteria had any effect on the rate of translation overall.

These proteins move around in a cell crowded by thousands of different proteins and must find their target efficiently. Thus, the crowding can change the rate of the cellular processes. To verify this hypothesis, I changed the cellular crowding by changing the growth conditions of the bacterial cells and measured the EF-Tu dynamics in the different conditions. Surprisingly, the dynamics hardly changed even though the overall translation rate changed significantly. This showed that the translation rate was independent of the motion of EF-Tu in a cell [8].

Bacterial resistance to antibiotics is one of the global challenges we face. Many scientists are doing research to develop new means to kill bacteria. I wanted to contribute to this effort with my knowledge of the Translation process. I started doing experiments to understand how different antibiotics or antimicrobial peptides affect this process. As I stated, translation is an extremely important process which occurs in every cell. Stopping this process can lead to bacterial death. To stop bacterial infections, scientists have developed numerous antibiotics to target the translation process. But our understanding of their effect isn't perfect. Thus, I studied a few such anti-bacterial materials to understand their effect on EF-Tu and ribosome dynamics.

6.5 What is the significance of my research?

Translation is one of the most important processes occurring in a cell: it determines which proteins will get produced in what quantity. These proteins, in turn, contribute to the proper functioning of the cell or the organism itself. Thus, it is important to understand how Translation occurs. Even though much research has been done to understand the translation process, our understanding of it is far from perfect. Also, most of these studies were done *in vitro*, which might not represent the actual picture. My findings shed light on this important process. I have reported how fast the EF-Tu – Ribosome interaction can occur and why the

translation process is very efficient. I also show how different the actual rate can be compared to the *in vitro* experiments.

The other part of my research was focused on understanding the difference in the effects of different anti-bacterial agents on the translation process. A lot of research is being done to produce different anti-bacterial drugs, but their exact effect is unknown. It is important to understand how different physical characteristics of these drugs – like structure, if they can or cannot function in water (called hydrophilicity or hydrophobicity, respectively), etc. - can lead to very different effects. If we can understand how different physical characteristics of drugs create specific effects, we can optimize those drugs to make them more efficient. By studying these characteristics and their effects, we may also be able to discover unknown steps in the translation process. I found one such step in the EF-Tu binding process, which is important in the overall mechanism.

Understanding processes like Translation is vitally important because this knowledge paves the path for drug development. Much research centers on treating bacterial infections, and as mentioned above, many of the drugs lose their efficacy in a short period of time. Thus, newer drugs are developed to target other functional aspects of the bacterium. By understanding these vital processes, we can develop newer targets to combat bacterial infections. Also, understanding the mechanism of current antibacterial drugs can help us optimize their functionality, increasing their antibacterial potency.

6.6 References:

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