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.

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Functional mapping of the components of the *E. coli* translational machinery

By

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Spying on the bacteria

The Good, the Bad and the Ugly

The word “bacteria” often has a negative connotation. People generally think of bacteria in the context of infectious diseases and consider them to be villains that need to be eradicated from the face of earth. However, a world without microbes is not only uninhabitable but also unachievable. Most bacteria are harmless or even beneficial to us. Our human bodies host trillions of bacteria that not only help digest our foods but also help protect us from diseases. Some of our favorite foods such as cheese, wine, yoghurt, pickles are the result of hard work done by bacteria. Owing to the simple machinery of bacteria, scientists modified them to be used as “micro-factories” for production of insulin, vaccines, other life-saving drugs and even jet fuel.

Why study bacteria?

Bacteria are single celled organisms with simple internal structure. Their simplicity, compared to other organisms, makes them suitable study subjects to answer fundamental questions related to cell functioning. A study of various aspects of bacterial cell functioning can help us design antibiotics that target harmful, pathogenic bacteria. These bacterial studies also enable us to manipulate the bacterial cells to carry out functions that are beneficial to us.

One of the most well studied bacteria is the rapidly growing *E. coli*, which divides every 20-40 minutes. Every organism contains a genome, which is like an “instruction manual” for that organism. This genome contains information and instructions for the operation of every single component of the organism. This instruction manual is photocopied and passed down to

the successive generations. The *E. coli* genome is nearly 1000 times smaller than the human genome, making it easier for scientists to analyze.

One reason we study *E. coli* so carefully is because the basic biological principles learned from experiments on *E. coli* are generally applicable to other organisms. French scientist Monod famously noted, "What is true for the *E. coli* is true for the elephant." Many of the genetic properties that govern *E. coli* are true for us.

Even though *E. coli* is a very well-studied organism, we still do not understand several aspects of its internal workings.

Understanding the inner workings of *E. coli*

If you were asked to analyze how a mechanical clock works, a reductionist would separate out its various components (gear trains, oscillators, springs) and then put them back one by one to understand how these different parts interact with one another. The overall behavior of the clock is determined by the arrangement of the different pieces of the clock as well as the interactions of the tiny pieces with each other.

Proteins are fundamental entities essential for the survival and growth of all biological cells, including *E. coli*. Their interactions result in a wide variety of protein complexes that serve as the basis for how cells function. Scientists extract proteins out of the cells and study protein interactions physically and biochemically *in vitro* (outside the cell). Most of these studies, however, cannot replicate the complexity proteins encounter in the cellular environment. Scientists find it extremely difficult to recreate the complicated cocktail of biomolecules that comprise the natural milieu of living cells.

E. coli is a much more complicated system than a clock. Its properties arise from a network of interactions between different components as well as with the surrounding environment. *E. coli* is more than the sum of its parts. The properties of any protein present in an *E. coli* are not equivalent to the sum of properties of individual amino acids that constitute the protein. The usefulness of a protein depends not only on the sequence of amino acids, but also on its three dimensional structure and environment. A reductionist approach may therefore not properly account for properties of a complex system like *E. coli*. To understand the biological complexity of *E. coli*, we need to investigate both the individual components (proteins, lipids etc.) and how these components interact with one another on a system-wide level. Scientists are therefore making efforts to study protein interactions in the natural context of living cells.

The network of protein interactions govern the interior architecture of *E. coli*. Two proteins that are located near each other in a cell may be engaged in the same process, or bind to a common element, or share sites of production, degradation or action. On the other hand, if two proteins avoid each other in a cell, investigating their spatial localization can give us information about physical and biochemical processes that lead to their sequestration. We can look at how different components are organized inside *E. coli* and obtain information about communication between different components of the same process as well as communication between different processes. How instructions from the genome govern the internal architecture of the cell is one of the open questions in biology.

Peeking into the internal architecture of bacteria

Antonie van Leeuwenhoek, a Dutch scientist, was the first person to observe different kinds of bacteria under his handcrafted microscope. More improved microscopes enabled us to take a closer look into the bacteria. Microscopy images revealed that bacteria lack any internal

organization or structural features. This is in stark contrast to other plant and animal cells where structures called organelles organize different cellular processes into different compartments of the cell. Based on this major difference, scientists categorized organisms into prokaryotes (such as bacteria) and eukaryotes (such as fungi, algae, plants, and animals including humans).

Bacteria were viewed as bags of disorganized molecules devoid of any intracellular organization. On the other hand, eukaryotic cells could be compared to our houses. Our houses have areas designated for different activities: kitchen for cooking, bedroom for sleeping and so on. Similarly, eukaryotic cells have different cellular regions allocated for different functions.

The last few decades have seen tremendous developments in the field of microscopy and imaging. This has allowed us to study the interior of a bacteria in detail. Studies from the early 90s showed that different bacterial components exhibit unique spatial patterns. This was followed by a wealth of studies focused on spatial localization of various cellular processes in several different bacteria. Scientists realized that bacteria are not as simple as they previously believed.

Recent developments in microscopy enabled bacterial imaging

In the 1960s, Osamu Shimomura, who had an interest in studying bioluminescence (the process by which animals emit light, e.g. fireflies), isolated a bioluminescent protein from a jellyfish. This protein gives off green light when illuminated with UV (ultra-violet) light, by a process called fluorescence. He named this protein the green fluorescent protein (GFP). Since then, scientists have discovered and modified many variants of the members of the "GFP family" of proteins.

GFP and its other variants basically serve as "spies" that scientists could introduce into the cells. GFP could be fused to any protein of interest without affecting the function of the

protein. The green fluorescent signal from a labelled protein maps its cellular location. These GFP-like proteins allow us to monitor in time and space an ever-increasing number of phenomena like gene expression, protein localization and dynamics, protein-protein interactions, cell division, chromosome replication and organization and intracellular transport pathways in living cells and organisms.

GFP revolutionized our ability to dissect mechanisms in *E. coli* and investigate its internal structure using microscopes. The discovery of GFP and its modification to tag proteins of interest provided a non-invasive method of looking at proteins in their natural environment. A fundamental set of physical laws govern the highest achievable point-to-point resolution that can be obtained with an optical microscope. These resolution limitations are referred to as the "diffraction barrier", which restricts the ability of optical instruments to distinguish between two objects separated by a lateral distance less than ~ 250 nm, nearly $1/400^{th}$ of human hair diameter. *E. coli* are rod-shaped cells that are 900 nm in diameter and 2-8 μm (~ $1/20^{th}$ of human hair diameter) in length. As a result, traditional microscopy methods with a resolution of ~ 250 nm were ill-suited for studying the inner workings of bacteria.

Scientists have modified some members of the GFP family to be "photoactivatable". We need two light sources to image these "photoactivatable" molecules. These molecules undergo chemical conversion when we shine a low power "activation" light at them. We then employ a high power laser to image only the activated molecules until they bleach. A picture of all of the photoactivatable proteins in the entire cell is developed by multiple rounds of imaging. This principle forms the basis of PALM (Photoactivated Localization Microscopy), whose discoverers were awarded the Nobel Prize in 2014. PALM has a "crowd control" feature that enabled us to image single molecules in live bacterial cells. Single molecule imaging in living cells provides us

with a wealth of information. In addition to providing very precise information about the location of an individual protein in a cell, PALM allows us to follow the protein as it is moving. We can think of PALM as a camera that not only provides static pictures of protein location but can also provide us with videos of that protein moving.

Importance of studying single molecules and their motion

Averages can be misleading at times. Just looking at averages, one could conclude that every person on this earth is half male and half female. Only by looking at individuals does it become obvious that they have different gender identities. In biological studies, different molecules behave differently and experience different local cell environments. Different cellular processes do not start and stop at the same time. In such situations, ensemble measurements can lead to the same sort of problem. Measurements that capture only the average of a population of molecules blur the differences between individual molecules, and important information is lost. One of the reasons for exploring single molecules is to discern whether there is different behavior from one copy of a molecule to the next. For some biological questions, single-molecule studies are a necessity. With the new PALM microscopy method, we can perform single molecule experiments on proteins in their natural environment.

We can use PALM to determine how fast a molecule is moving. This in turn can help us sort molecules into groups of different mobility. One can also identify in which areas of the cell a protein diffuses slowly and in which regions of the cell it diffuses faster. Correlating the motion of a protein with its intracellular location can give us an idea about the function of the protein. For example, if we knew GPS locations of all the members in the Chemistry department of UW Madison, we could potentially make educated guesses about the roles of each member in the department. If members spend most of their time in business offices, they are most likely

employees of the business office. If some members spend time in different classrooms during the day, they are more likely to be students of the department. Similarly, if a protein localizes to the region of the cell where DNA exists, it is probably a DNA binding protein. On the other hand, if a protein localizes to the membranes of the cell, it is likely to be a membrane protein engaged in transportation of molecules into and out of the cell, and communication between cells. This information on the motion of single proteins, in combination with the vast number of *in vitro* experiments done on proteins in *E. coli*, helps us determine the function of proteins in different regions of the cell.

A protein molecule could switch from very mobile → immobile →very mobile and vice-versa during the course of its motion. The mobility of a molecule is related to its role in a cellular process. When a protein is bound to its target site, it is immobile; and when it is searching for its target site, it is very mobile. We can measure the time a protein spends bound to its target by measuring the amount of time it stays immobile.

What did I work on during my PhD?

During my PhD, I relied on these single molecule tracking methods to quantitatively study the translational machinery of *E. coli*. There are many processes that take place in cells that are essential for life. Translation is one of the most important cellular process in all organisms. It is the process by which cells make proteins for their survival. The cells spend a lot of energy making these proteins. Cells employ ribosomes as the protein builders.

As a part of my thesis, I have worked with *E. coli* in two different growth conditions; fast and slow. In the fast growth conditions, I provided *E. coli* with nutritious food that allowed them to grow rapidly (dividing every 50 min). In the slow growth conditions, *E. coli* were starved

which led them to grow slowly and divide every 150 min. We were curious if ribosomes would be distributed differently in a happily growing *E. coli* and a starved *E. coli*. I labelled ribosomes with YFP (yellow fluorescent protein, a member of the GFP family). In addition, I labelled the DNA of the cell with a dye called Sytox Orange. This allowed me to see the location of the fluorescent ribosome molecules with respect to that of DNA. In Fig. 6.1, I have shown the location of ribosomes and DNA in the two differently grown *E. coli*. Similar to earlier studies, we observed that in fast growing *E. coli*, there are two regions (nucleoids) where most of the DNA is located. In contrast, in slow growing cells, there is a single nucleoid region. The ribosomes and DNA avoid each other despite the difference in growth conditions.

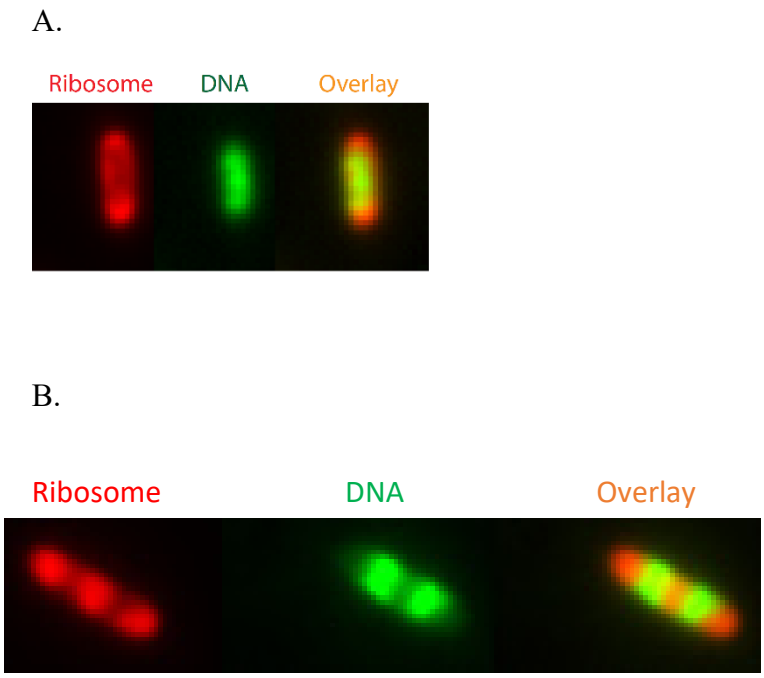

Fig. 6. 1 Imaging of ribosomes labelled with YFP and DNA labelled with Sytox Orange in two different growth conditions. The composite image shows that ribosomes and DNA avoid each other in *E. coli*. **(A)** Slowly growing *E. coli* **(B)** Rapidly growing *E. coli*

The ribosomes that are engaged in protein building (translating ribosomes) are less mobile compared to the ribosomes that are looking for sites to begin protein building (searching ribosomes). Using PALM, I was able to distinguish the translating ribosomes and the searching ribosomes. Our comparison of how many ribosomes are engaged in translation in these two different kinds of cells revealed that there is a slight decrease in the number of translating ribosomes in slow growing *E. coli*. One possible explanation for this is that the starving *E. coli* require fewer proteins to survive.

We were able to map ribosomes in different regions of the cell based on their functions. We found that the majority of translating ribosomes are located outside the nucleoid region, which could indicate that most of the translation in cells occurs in regions outside the nucleoid. The ribosomes that localized to the nucleoid region of the cell are searching for sites to initiate construction of a protein. We were able to estimate timescales of exchange of ribosomes between different cellular regions.

How can my research benefit the public?

In my graduate research, I have used cutting edge tools to obtain a detailed and quantitative understanding of the translational machinery in *E. coli*. We hope that my graduate research work using the model bacteria *E. coli* could open doors for possible development of new antibiotics targeting the translation process. Human biology encompasses much more than what goes on in human cells, as there are at least as many bacterial cells as human cells on and in our bodies. *E. coli* may not be the most prevalent bacteria in our body, but we hope that our work

can be extended to other bacteria that are critical to human biological processes.