

# Communicating Research to the General Public

---

At the March 5, 2010 UW-Madison Chemistry Department Colloquium, the director of the Wisconsin Initiative for Science Literacy (WISL) encouraged all Ph.D. chemistry 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, state legislators, and members of the U.S. Congress.

Ten Ph.D. degree recipients have successfully completed their theses and included such a chapter, less than a year after the program was first announced; each was awarded \$500.

WISL will continue to encourage Ph.D. chemistry students to share the joy of their discoveries with non-specialists and also will assist in the public dissemination of these scholarly contributions. WISL is now seeking funding for additional awards.



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.

**UW-Madison Department of Chemistry**  
**1101 University Avenue**  
**Madison, WI 53706-1396**  
**Contact: Prof. Bassam Z. Shakhshiri**  
**bassam@chem.wisc.edu**  
**www.scifun.org**

January 2011

**APPENDIX 1**

INTERRUPTING BACTERIAL CONVERSATIONS:

DESIGNING CHEMICALS TO CONTROL QUORUM SENSING

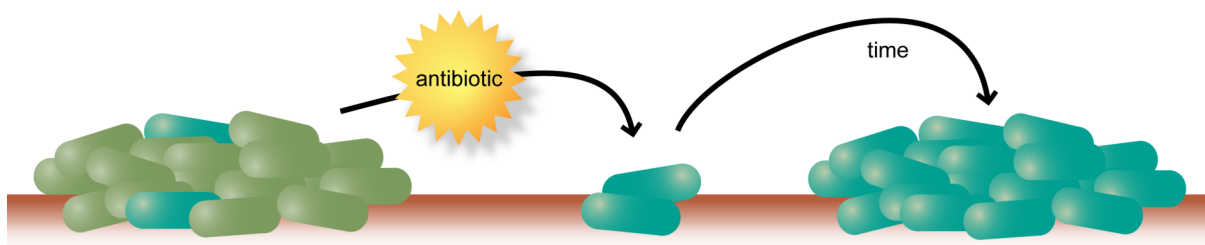
IN *PSEUDOMONAS AERUGINOSA*

*By Margrith Mattmann*

\*This chapter was prepared for the WISL \$500 Graduate Student Awards to Promote Communicating Chemistry, announced by Professor Bassam Z. Shakhashiri.

### What doesn't kill you makes you stronger

Antibacterial resistance is a growing problem. The most common hospital-acquired bacterial infections have significant resistance to some of our most powerful drugs, and already some strains are beginning to surface that cannot be killed by antibacterial therapy we have access to. Yet bacteria themselves cannot be blamed for their remarkable powers of survival; much of their resistance has developed because regular treatment with antibacterials provided bacteria with many chances for the fittest to survive against even our strongest drugs.



**Figure 1:** Development of resistance. When a mutation in normal bacteria (green) leads to the ability to resist antibiotics (blue bacteria), these organisms will survive antibiotic treatment and grow into a group of drug-resistant bacteria.

Like all living things, bacteria have DNA that acts like a code to help determine who they are and what they can do. As they eat, grow, and reproduce, bacteria make copies of their DNA to pass down to their children. Sometimes, as DNA is copied, mistakes are made and changes called *mutations* occur at random. These mutations can be lethal, have no effect, or provide some advantage, even if these effects are not immediately clear. In the presence of some kind of external pressure like extreme temperatures, toxins, a new living environment, or antibacterial drugs, organisms who by chance have been given some kind of advantage will be better suited to continue to eat, grow, and reproduce. As the non-mutated and less-

well suited organisms die off, these mutations become part of the DNA that is passed through the generations. Greater and more frequent threats will enhance the rate at which mutations develop. Resistance is one of these mutations (**Figure 1**). As this process repeats itself over time, bacterial populations can develop that are resistant to multiple drugs. For example, *Multi-drug Resistant Staphylococcus aureus*, or MRSA, is now one of the most common hospital-acquired infections and its resistance against our most advanced antibiotics makes it one of the most dangerous.

In addition to developing resistance to powerful antibiotics, bacteria have an arsenal of weapons to ensure their survival. Infectious bacteria often produce toxic substances in order to compromise the immune system of their host. They can surround themselves with impenetrable defensive *biofilms* that protect them from the host's immune system and antibacterial treatment. The only advantage we currently have against such infections is to use antibacterial drugs to clear the bacteria at early stages of infection or use more powerful doses against established disease. This may treat the immediate infection but in the long run contributes to the growing problem of antibacterial resistance.

An alternative approach to treating bacterial infection is being developed in which the goal is not to kill the bacteria outright but to make bacteria less *virulent*, or toxic. Crippling the bacteria's defensive and offensive strategies should make them easier to kill using relatively weak antibiotics. By limiting the perceived threat to the bacteria, this approach slows the development of new antibacterial resistance and provides a novel direction for the development of effective therapeutics.

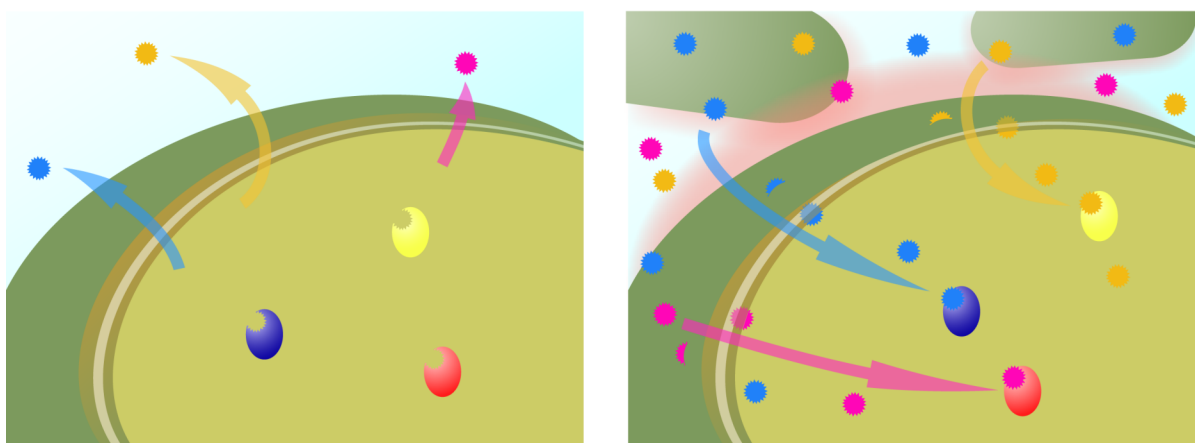
### Group behavior: Quorum sensing

For the single bacterium, there is little advantage in launching a virulent attack. It requires a certain amount of energy to produce toxic products and build defenses, and this energy is required first and foremost for eating, living, and reproducing. A single bacterium would also find it difficult to withstand a host's immune defenses. To address these challenges, bacteria wait until a larger group assembles, at which point they change their behavior patterns and act as a unified group. To do this, bacteria rely on a robust communication system. Since the 1970s, bacteria have been recognized as social creatures that communicate with each other in a process called *quorum sensing*. Using a set of chemicals as a kind of language, high populations of bacteria can coordinate complex group behaviors. This social behavior should be quite familiar, especially to those who have encountered the “mob mentality” often pervasive in groups of sports fans (**Figure 2**). Alone, even the most extreme sports fanatic seems rather unobtrusive. Yet in the presence of the right chemicals (like beer) and increasing numbers, this group of sports fans acts together in ways the individual would not.



**Figure 2:** Group behavior. The group of UW football fans, given the appropriate chemical stimulant and the power of increasing numbers, will act in ways the individual would not.

Biologically, quorum sensing is a specific and often complex process (**Figure 3**). Bacteria produce chemicals for communication along with corresponding *receptors*. This receptor is a protein that is usually *specific* for a given chemical signal, and functions best when its corresponding signal matches up and binds with it. As the population of bacteria grows, the concentration of signal molecule increases, and the signals collecting in the environment begin to diffuse into the bacteria.



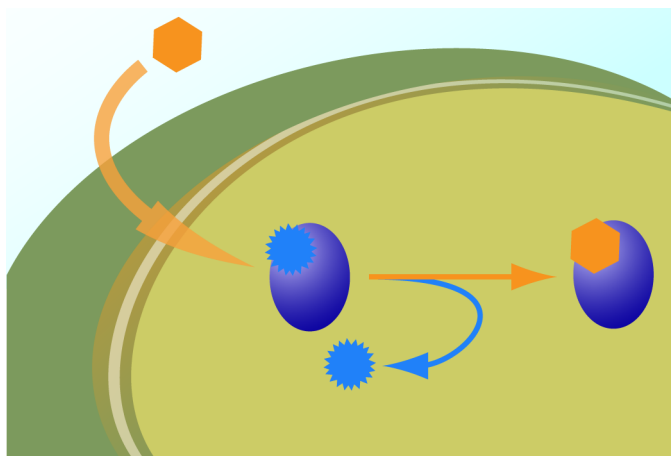
**Figure 3:** Quorum sensing. The bacteria are green, chemical signals are represented as stars, and protein receptors are shown as ovals. Both images show a magnified slice of a single bacterium, where the lighter color represents inside the cell and the darker color represents the cell membrane. **Left:** Low bacterial population. Chemical signals are produced and exit the bacterial cell. **Right:** High bacterial population. Chemical signals now diffuse back into the bacterial cells, where they bind with their associated receptor proteins. This initiates a set of group behaviors, represented by a reddish glow.

This is the feedback the bacteria have been waiting for. As they bind with their specific receptors, the signals trigger changes in bacterial behavior. As a group, the bacteria do more than simply eat, live and reproduce. The group initiates defenses like the production of biofilms, and conducts offensive tactics like the production of toxins. This activity, in addition to the problems associated with evolved resistance, makes bacterial infections difficult to treat, and helps explain why they are so dangerous. In humans, bacterial infections are usually detected upon perception of symptoms. By this time, quorum sensing is

in full swing and the bacteria have already formed a virulent and well-defended group. It is clear that being able to prevent bacteria from initiating these group behaviors or disrupting them once they have been established represent important targets for new therapeutic approaches.

### **Interrupting the conversation: Our research strategy**

My research is conducted under the supervision of Professor Helen Blackwell. A large portion of research performed in her laboratory is devoted to the interception of quorum sensing behavior in several bacterial species. We have identified the receptor proteins as targets. Our hypothesis is that we can make specific changes to the natural communication signals and develop artificial signal molecules that will bind specifically to our target protein of choice. If they bind, we might be capable of controlling bacteria by exploiting their own communication pathways (**Figure 4**).



**Figure 4:** Anti-quorum sensing strategy. Artificial signal molecules (orange) are added to the bacteria. These signals then compete with the natural signals (blue) for the receptors. This new signal:receptor complex is then unable to perform in the same way as the natural complex, which disrupts QS and group behavior.

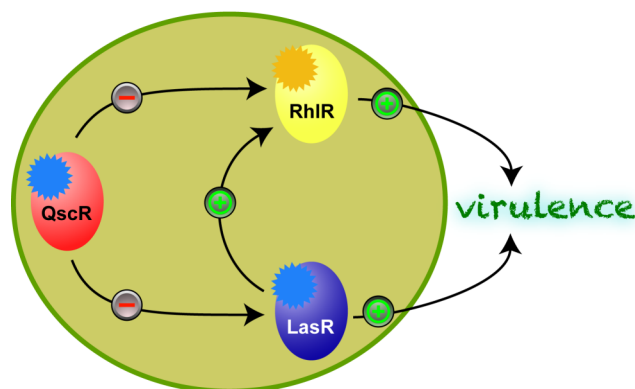
In designing these signals, it is important to remember that many bacteria use several different receptors, and therefore several different signals, to coordinate their group behavior. Therefore, our signals must not only bind to their receptors at least as well as the natural signals, they must also be very selective for the intended target receptor protein over any other proteins in the bacterial cell. Previous Blackwell group members have developed straightforward ways to make these signals quickly and in high purities. Most of our current efforts, therefore, are devoted to testing our signals to see how well they bind to their receptors. Signals that excel in doing this are further tested to see whether they are capable of changing the behavior of groups of bacteria. I have applied our strategy to the plant, animal, and human pathogen *Pseudomonas aeruginosa*.

### ***Pseudomonas aeruginosa***

*Pseudomonas aeruginosa* is an opportunistic pathogen and, along with MRSA, is one of the most common hospital-acquired infections. This pathogen commonly infects burn victims and chronically ill patients, including people with cystic fibrosis for whom such an infection is often inevitable and fatal. *P. aeruginosa* represents a clinically relevant model system of a bacterial species that uses a complex quorum sensing circuit to coordinate group behavior. Quorum sensing in this organism is controlled by three receptors (**Figure 5**). Two of these receptors seem to use the same signal molecule, while the signal for the third receptor is distinct. Each receptor bears a name related to the role it plays within this complex quorum sensing circuit. The LasR receptor was the first receptor identified in *P. aeruginosa*. It is the main regulator of the production of elastase, which is the enzyme responsible for degrading elastin, a major connective tissue found in the lungs. This receptor



activates RhlR, which mainly regulates the production of rhamnolipids. These greasy molecules are exuded from the bacteria and provide a slippery surface upon which the bacteria can easily travel and spread infection. Both LasR and RhlR also cause the production of many other virulence factors, including toxins and biofilm. *P. aeruginosa* also maintains negative regulators of quorum sensing that inhibit group behavior. One of these is QscR, the quorum sensing control repressor protein. This receptor uses the same signal molecule as LasR and, through some as yet unconfirmed mechanism, represses the activities of RhlR and LasR. By using this highly developed communication system, *P. aeruginosa* can adapt to a variety of living conditions and coordinate many virulent group behaviors.



**Figure 5:** Simplified schematic of the quorum sensing circuit in *Pseudomonas aeruginosa*. Ovals represent receptor proteins, and stars represent the communication signals associated with each receptor. QscR represses the activity of RhlR and LasR. Meanwhile, LasR positively regulates RhlR, and together these two regulators produce virulent group behavior.

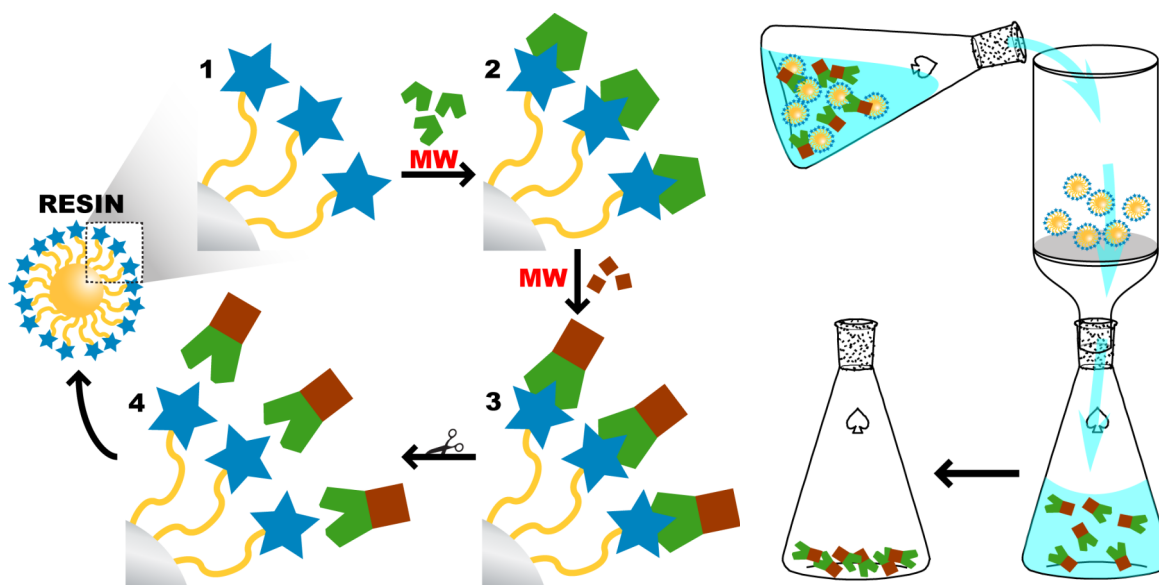
Because each of these receptors requires a small chemical signal for activity, they represent interesting targets for our approach. By taking advantage of the selectivity of these receptors, as well as their relationships to each other and to the production of virulence, we can develop artificial quorum signals capable of disrupting group behavior in *P. aeruginosa*. There are two methods we can use to accomplish this. First, we can focus on identifying artificial quorum sensing signals capable of selectively activating or inhibiting these

receptors. Second, since LasR and QscR share the same signal, it may be possible to develop artificial signals that have *shared* activity between receptors. This would provide us tools capable of potent control of quorum sensing with a single molecule. The combination of these approaches provides tremendous versatility in designing custom “cocktails” of signals for control of *P. aeruginosa* group behavior. For example, the quorum sensing pathway indicates that in order to silence virulence, we must deactivate LasR and RhlR and activate QscR. We could find three compounds selective for their target receptors and administer them together. Or, rather than using three separate compounds, we could employ a single compound that can inhibit LasR and RhlR *and* activate QscR. I have focused my project on identifying artificial chemical signals that allow exquisite control over group behavior. These cocktails not only represent platforms for future therapeutic development, but also provide insight into how *P. aeruginosa* coordinates group behavior by balancing the activity of its three receptors.

### **Using solid phase chemistry to make compounds**

Chemists use different kinds of chemistry to make particular types of molecules. They excel at making a single, complex compound very carefully; alternatively, they can make libraries of tens or even hundreds of more straightforward compounds simultaneously. Because the signal molecules we are building are relatively simple in structure, we employ this latter approach. The method we use is different than what many people think of when they imagine a chemistry lab with test tubes and beakers bubbling away. This chemistry is performed in small vials rather than in test tubes and beakers, and if we have done everything right, when we are finished we produce a small amount of powdery compound: perhaps

enough to cover your pinky fingernail. The synthesis is straightforward, efficient, and produces compounds pure enough we can use them right away in biological experiments without purifying them further. I can imagine the molecules I would like to make on Monday and begin using them in my biological experiment of choice by Friday, which is a conveniently quick turnaround.



**Figure 6:** **Left:** General concept of solid phase synthesis. Beginning with a resin, chemical building blocks are added stepwise until the desired chemical is built. In each step, the reaction mixture is placed into a microwave (indicated by a red **MW**) where the heat causes the reaction to take place much more quickly than it would otherwise. In the last step, a cleavage reaction cuts the desired molecules from the resin. **Right:** The completed reaction mixture containing resin and released compound is filtered, and the final product is obtained after drying.

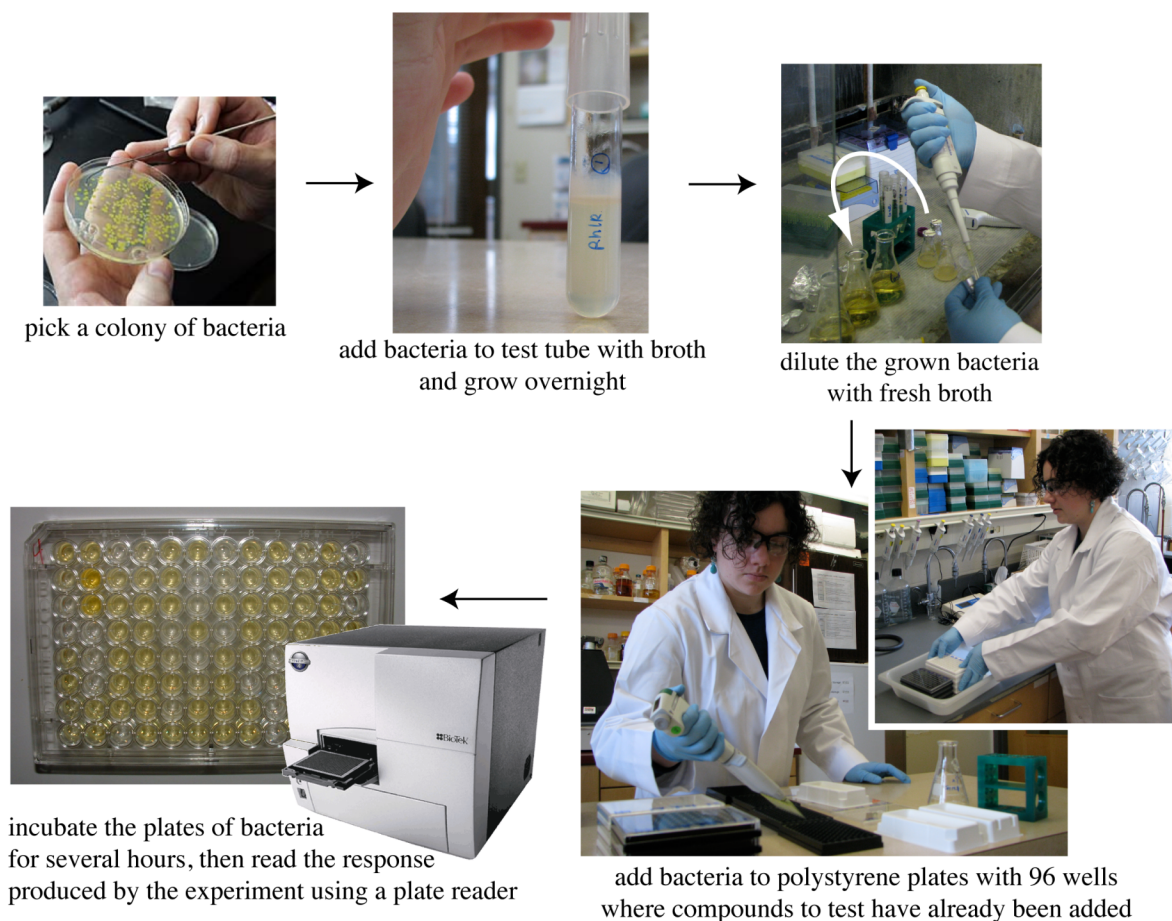
Our synthetic method of choice is called “solid phase synthesis” because we build our molecules on top of sand grain-sized pieces of plastic. The easiest way to explain our method of synthesis is to imagine sticking the parts of the molecule one by one on top of the resin, like clicking Lego blocks onto one another on the grain’s surface. In **Figure 6**, a single grain of resin is depicted on the left in a highly magnified cartoon. For each compound we wish to make, we need to dissolve about a half a teaspoon of dry resin in a vial with some solution.

In library synthesis, we can set up as many reactions as we want to, so it is important to keep track of which vial corresponds to which reaction. Each grain of resin is covered with reactive areas, which are shown as stars in part 1. In each step, we dissolve the resin in an activated solution with new building blocks that when put together make up the compound we are interested in. This reaction mixture is placed into the microwave where the reaction can take place very quickly and attach our building blocks to the reactive surface of the resin, as shown in part 2. This process can be repeated as many times as necessary to build the signal compound. Once the signal is built, we dissolve the now highly decorated resin in some very harsh chemicals that remove the compounds from the resin and release them into the solution. The final step of our synthesis highlights the efficiency and simplicity of this method. The reaction mixture, which contains resin as well as the compound we have just released from the resin in the cleavage step, is poured over a filter. The resin is collected in the filter, while the dissolved product compound passes through the filter in the solution. After evaporating off this solution, only pure compound remains, usually as a white solid or powder. Since we began with about a half a teaspoon of resin and have collected molecules that have been released from the entire surface of each grain of resin, we should have collected about enough of each pure compound to cover the surface of your pinky fingernail. This is more than enough to keep us busy with biological experiments for months.

### **Testing the artificial signal compounds: Biological assays**

Once we have designed and synthesized libraries of artificial signal molecules, we must test them to see whether they are capable of controlling bacterial group behavior. There are several different kinds of tests that can be performed to examine this. Several Blackwell

group members have developed ways to test the effects of our compounds on *Pseudomonas aeruginosa*. We have evaluated the ability of our compounds to affect group behaviors like the production of defensive biofilms and toxins and we have examined the survival of infected plants. Some of our compounds have been sent to collaborators at other universities where they have been used in biochemical tests in hopes to learn what our molecules are doing inside the bacteria to cause the changes in activity we observe.



**Figure 7:** General assay setup. See text for details.

Generally, bacterial assays follow a similar procedure, which is illustrated in **Figure 7**. Bacteria are grown in nutrient-rich broth in a test tube or small beaker overnight. The next

morning, these bacteria are diluted with fresh broth and allowed to grow again. This culture is introduced to our compounds by way of 96-well plates. Before I begin a biological assay, I set up the plates so that each well contains a certain concentration of artificial signal molecule. Now, when the bacteria culture is added to the plates and allowed to grow again, the bacteria will react to the presence of the compound. After a time, I can analyze this effect in one of several ways. Usually, this involves using our plate reader, which is an instrument that can measure absorbance, fluorescence, or luminescence. I can set the plate reader to measure whichever readout corresponds to the bacterial process that is affected by my compounds.

Our tests so far have confirmed our hypothesis and have shown that our artificial signal molecules can affect the activity of the receptors we target. We have been able to use this control to modestly attenuate *Pseudomonas aeruginosa* group behavior. We are a long way off from developing the next generation of therapeutics, however. There is still a lot to learn about the precise mechanism by which our compounds act, and we are always looking for new compounds and new combinations of compounds will give us the tailor-made control we seek for each organism of interest. Even so, the research performed in our lab and by others in our field provides tremendous insight into bacterial communication and demonstrates an emerging paradigm shift for the treatment of bacterial infections.