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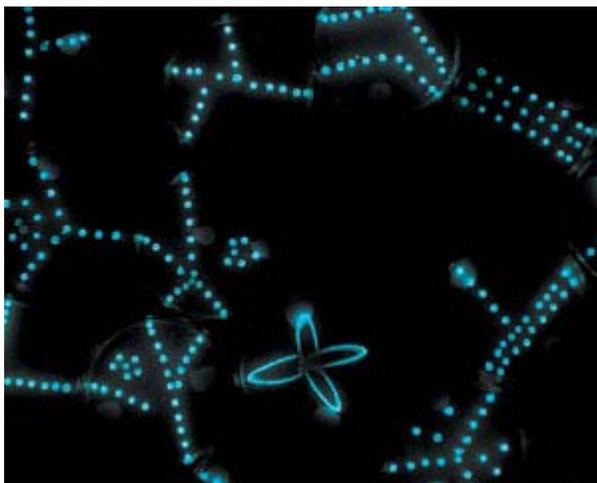
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Bacterial Conversations

Using a chemical language, bacteria coordinate everything from infection to plaque buildup

[Sarah Everts](#)

Hundreds of species of bacteria in the plaque on your teeth are currently having a conversation in a language composed entirely of chemical words. This may be a hard idea to swallow, but bacterial conversations by way of molecules are ubiquitous. Bacteria are chatting in the sludge of your kitchen sink, in your gut, at the bottom of jet fuel tanks, on your contact lenses, in the roots of plants, in the lungs of cystic fibrosis patients, and in persistent infections found in contact with medical implant devices like pacemakers.



B. Wittstock/MSU-Bozeman Bioglyphs Project
Light Chatter When *Vibrio* bacteria get together, their chemical conversations induce the expression of light-producing proteins.

These bacteria are talking about when to infect a host or when to shack up in slimy, protective habitats known as biofilms that keep nutrients from diffusing away and antibiotics from diffusing inside. Often the bacteria are arranging to trade genes for such useful traits as antibiotic resistance or how to catabolize a new energy source. These conversations allow bacteria, which are single-celled organisms, to turn on genes that synchronize group activities, thereby enjoying some of the perks of multicellular life.

"Bacteria have invented a way to coordinate group behavior so that they can get things they couldn't get on their own," says [Bonnie L. Bassler](#), a Howard Hughes Medical Institute investigator and professor of molecular biology at Princeton University.

"To a first approximation, genes turned on as a consequence of chemical communication do no good to bacteria in isolation," Bassler says. "A single bacterium making light is not particularly useful. Luminescence, virulence, and exchanging DNA—these are all things that work better if the group carries them out in unison."

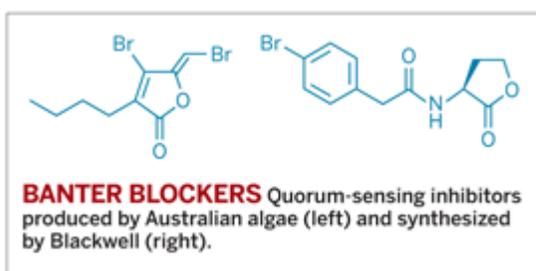
These bacterial conversations are called quorum sensing, and they occur, as you might expect, when a quorum of bacteria are in close proximity. The language is made up of chemicals called autoinducers, which are continually released at low levels into nearby surroundings, like a chemical beacon in the dark. Bacteria also have receptors that can detect the autoinducers, so they know when other bacteria are sending signals. When the bacterial population density increases—or when the concentration of these autoinducer compounds rises past a certain threshold—a selection of genes are turned on or off, helping the bacteria switch between solitary and group activity.

Many quorum-sensing molecules facilitate discussion among bacteria of the same species and strain; others permit cross-species cross-talk. And not all conversations between bacteria are altruistic; in fact, some are downright nasty. Bacteria use their repertoire of quorum-sensing chemicals to suit their long-term goals, be that pathogenesis or symbiosis. "You use the same words in a civil conversation as you do in an argument. For both bacteria and humans, it's the context and how

loudly you speak that determines whether it's an argument or a cooperative discussion," says [Edward G. Ruby](#), a microbiology professor who studies quorum sensing at the University of Wisconsin, Madison.

Regardless of the conversational tone, scientists are trying to listen in, so they can understand the rules of engagement among microscopic organisms. The hope is that, by eavesdropping on bacterial chatter, researchers will be able to design inhibitors to thwart discussions among some of the more pathogenic organisms.

Luminescent bacteria known as *Vibrio fischeri* that populate marine animals like the tiny Hawaiian bobtail squid led to the discovery of quorum sensing. Light emission is costly to the bacteria, and they only produce the necessary proteins when there are enough bacteria in close quarters to make substantial light, such as in the confines of the squid's light organ. The bacterial luminescence helps the squid mate and hunt. In exchange, the bacteria get shelter and food.



In the early 1970s, microbiologists Kenneth H. Nealson and J.

Woodland Hastings at Harvard University set out to discover how bacteria like *V. fischeri* knew when to turn on bioluminescence. They found that the production of luminescent proteins is tied to an acyl homoserine lactone (AHL) that *V. fischeri* both release and detect. In close quarters, such as in a squid's light organ or a test tube or a biofilm, the bacteria divide until their numbers, and the concentration of AHL, rise to a certain level. When there are enough bacteria to produce substantial light—gauged by AHL concentration—*V.*

fischeri bacteria can agree to turn on the genes to produce the luminescent proteins. In this way, *V. fischeri* do not waste energy making light proteins when there aren't enough bacteria nearby to generate useful light for, say, a squid to hunt.

On the basis of their observations of luminescent bacteria, Nealson and Hastings first proposed the idea of autoinduction, or quorum sensing. The idea was mostly ignored, except when it was derided. "My biggest regret is not saving some of the review letters from our first papers," jokes Nealson, now a professor at the University of Southern California. "People just thought quorum sensing was, at best, an unusual exception in marine bacteria," he says. In the 1990s, "the breakthrough in acceptance finally came when others showed the same process was occurring in other species."

These other species include *Pseudomonas aeruginosa*, one of the nasty bugs that colonize the lungs of cystic fibrosis patients. *P. aeruginosa* uses a similar AHL to coordinate group invasion of a host. Next, researchers discovered a plant pathogen called *Agrobacterium tumefaciens* that uses AHLs to coordinate the development of tree tumors and also to initiate exchange of DNA between bacteria.

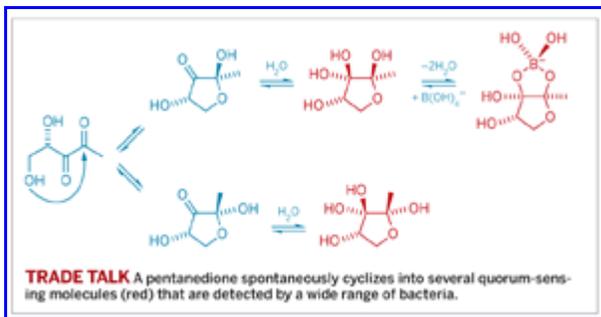
It turns out that AHLs are common chemical words among gram-negative bacteria—bacteria that have two sets of membranes surrounding their cytoplasm, like *Escherichia coli*. AHLs share a conserved homoserine lactone ring but have different acyl chains that range from four to 18 carbons, always even-numbered. Slight variations in the chain length and oxidation at the 3 position provide different gram-negative bacteria with distinctive quorum-sensing words. Particular bacterial species will produce a specific AHL that they use to communicate with their own kind. Currently, more than 75 species are known to use AHLs, and only 25 or so naturally occurring AHLs have been identified, so it's likely that cross-talk between species occurs.

All the gram-negative bacteria share an enzyme that builds the chemical lexicon from *S*-adenosylmethionine and the acyl side chain. Most AHLs freely cross bacterial membranes and are detected intracellularly by another protein called LuxR. When two LuxR proteins bind an AHL, they form a dimer that can bind to DNA and activate (or repress) target genes. Activated genes code for proteins that are involved in enabling virulence, producing toxicity, or even generating hydrogen cyanide.

But the lexicon is different in other types of bacteria. Gram-positive bacteria—those with just a single membrane, such as *Staphylococcus*, whose antibiotic-resistant strains are to blame for many persistent infections acquired in hospitals—like to socialize too. Instead of AHLs, the gram-positive bacterial language is composed of modified peptides. Ranging from five to 17 amino acids, these quorum-sensing signals are further modified by thiolactone rings, lanthionines, or isoprenyl groups.



Chris Frazee and Margaret McFall-Ngai/University of Wisconsin, Madison
Symbiotic Syntax The Hawaiian bobtail squid, whose symbiotic *V. fischeri* bacteria discuss when to luminesce, helped researchers decode quorum sensing.



[View Enlarged Image](#)

[Richard P. Novick](#), a microbiology professor at New York University Medical School, first identified these molecules in the mid-1980s, before the idea of quorum sensing was widespread. "It never occurred to us at the time that they were involved in bacterial communication," he says.

Novick knew that the molecules were important in exchange of DNA among bacteria, now recognized as a common outcome of quorum sensing. However, it wasn't until the 1990s when microbiologists began to appreciate the role of AHLs in gram-negative bacteria that Novick realized that his group had found the equivalent in gram-positive bacteria.

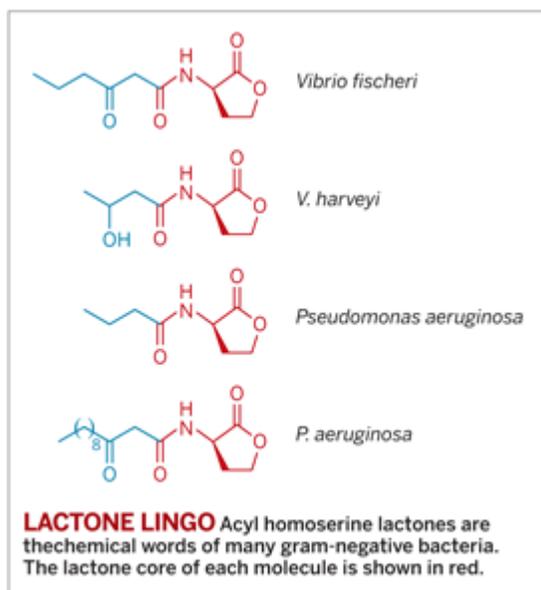
Instead of diffusing across membranes like AHLs, peptides are actively transported outside the cell and are detected externally by membrane proteins. Upon binding of the peptide, these membrane receptors dimerize and initiate an intracellular phosphorylation cascade that eventually activates quorum-sensing genes.

It is in *Staphylococcus aureus* conversations where a potent example of bacterial chemical warfare emerges. When *S. aureus* bacteria want to initiate infection, they first set up shop in a host, expressing proteins that help with external colonization and attachment. After a quorum of *S. aureus* is established, accumulation of autoinducing peptides activates genes for virulent proteins that help the bacteria invade a host. The four most common strains of *S. aureus* produce four slightly different peptides that potently inhibit other strains' receptors. The concentrations of the peptides—and which strain is growing fast enough—dictate who gets to chat and who is shut up. This means that the first strain to reach the highest population density is the only one able to establish quorum sensing and, consequently, virulence.

Quorum-sensing-based chemical warfare is not restricted to the intraspecies inhibition of *Staphylococcus*. Many strains of *Bacillus*, a family of pathogens that includes anthrax, also try to silence their competitors. These gram-positive bacteria secrete a lactonase enzyme that degrades gram-negative quorum-sensing chemicals by hydrolyzing the AHL ring. Likewise, *P. aeruginosa*, a gram-negative bacterium, has an AHL autoinducer that degrades into tetramic acid, which kills gram-positive competitors.

"It's a way for *P. aeruginosa* to keep other bacteria off its turf," says [Kim D. Janda](#), a chemistry professor at Scripps Research Institute who discovered this example of bacterial warfare.

Indeed, the range of chemical bantering and battling makes scientists extremely curious about the conversations occurring in a biofilm, the natural habitat of most bacteria and where different bacterial species coexist. One of the most common sets of genes turned on as a consequence of quorum sensing results in biofilm formation.



Courtesy of Janda
Janda



Montana State
University
Stewart

When a quorum is reached, many bacteria build and expel a complex variety of carbohydrates, proteins, and DNA that create a sticky matrix, and they do so for good reason. In biofilms, bacteria can isolate themselves near a food source and access a steady stream of nutrients. Bacteria in biofilms are a thousand times more resistant to antibiotics than bacteria in liquid suspensions, says [Philip S. Stewart](#), director of the Center for Biofilm Engineering at Montana State University. He thinks the resistance occurs because antibiotics are degraded by enzymes in the matrix, or because some bacteria enter a dormant, nonresponsive state.

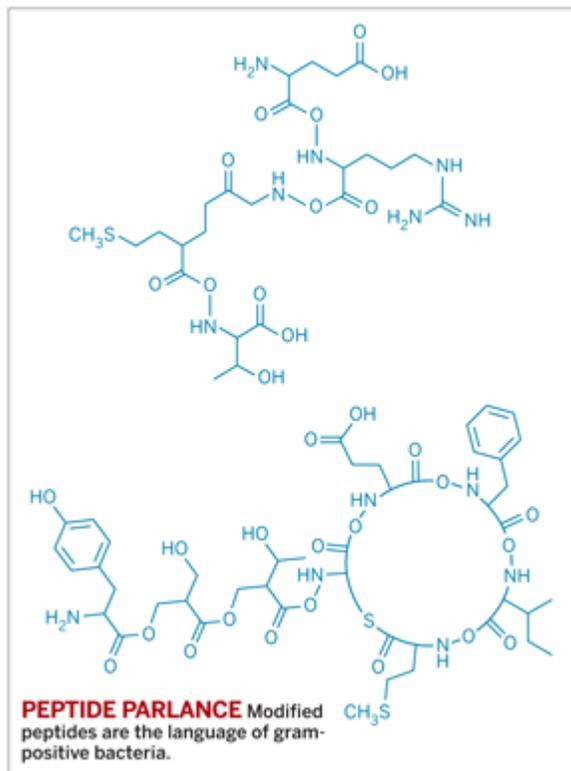
Because bacteria preferentially live in biofilms amid a variety of species, it may not be surprising that they also use a trade language. Many gram-negative and gram-positive bacteria sense each other's presence through a molecule called AI-

2. "When you are counting neighbors you probably want to know both what your kind is doing and whether your kind is in the majority or minority," says Princeton's Bassler, who first identified AI-2.

About five years ago, Bassler's group found what seemed to be a quorum-sensing circuit in a gram-negative bacterium that was independent of the typical AHL signals. They identified the protein receptor of the autoinducer but couldn't figure out the autoinducer's chemical structure because they could not purify it. "We kept getting this sludge," Bassler says. But since the autoinducer gene was present in "a who's who of bacterial pathogens, we obviously kept trying."

To solve the structure of the autoinducer, Bassler's group had to solve the structure of the entire receptor protein with the autoinducer bound. To their surprise, the autoinducer included an atom of boron, which is essentially nonexistent in most biological systems. "But after a while we realized that this actually made sense since the bacteria we were studying live in the ocean where there is lots of boron," says Bassler.

But how could a bevy of pathogenic bacteria—including *Salmonella*, which attacks the human gut, where there is no boron—be using this signaling molecule? What about conversations in dental plaque, also depleted of boron, where AI-2 is specifically required to create spatially organized biofilms and to initiate sugar metabolism?



It turns out that AI-2 is actually an interconverting family of molecules based on a pentanedione. Different bacteria recognize a variety of pentanedione derivatives, allowing them to sense bacteria both of their own kind and of others. Janda has since synthesized AI-2, which "was surprisingly difficult, given that it's only got a five-carbon backbone." Janda's group has also found that AI-2 can complex with carbonate, which is an important bacterial signaling molecule in dental plaques.

Although many bacteria both release and detect AI-2, some just produce it while others might just detect it. This inconsistency makes some researchers doubt AI-2's role as a quorum-sensing molecule. Others, like [Michael G. Surette](#), an associate professor of microbiology at the University of Calgary, in Alberta, call this phenomenon "eavesdropping." Some bacteria can sense the presence of others, respond to their presence by turning genes on or off, but not alert the other species of bacteria to their existence, Surette says. This is exactly what *P. aeruginosa* does in the lungs of cystic fibrosis patients. These patients have a genetic mutation that leads to defects in the ability of the lung to clear infections, leading to opportunistic colonization and biofilm formation by a dozen different bacteria, of which *P. aeruginosa* is a particularly harmful one. It can detect AI-2 molecules produced by normal throat flora, and this turns on genes that enhance its virulence. "It seems that *P. aeruginosa* is using AI-2 from the other bacteria to sense that it is in the right kind of host," Surette says.

More than just a bacterial social curiosity, quorum sensing is also a growing focus for researchers looking for new ways to control pathogenic bugs.

But controlling quorum sensing doesn't actually kill bacteria, a subtlety that some researchers say is actually advantageous, especially given the increasing incidence of bacterial resistance. The reasoning is straightforward: Since antibiotics are fatal, there is an enormous imperative for bacterial populations to evolve ways to evade death. Blocking quorum sensing just disarms the bacteria, which gets rid of the nasty activities but doesn't create as strong an evolutionary imperative for resistance.

"Blocking quorum sensing in pathogenic bacteria is not an antibacterial strategy—rather, it's an antivirulence strategy. You don't want to kill the bacteria, you just want to keep their bad behavior in check," says [Helen E. Blackwell](#), an assistant chemistry professor at UW Madison. "Even if you have compounds that inhibit virulence, you still need to clear the infection. However, this might require a lower level of antibiotics. So synergistic therapies are attracting a lot of interest."

Blocking bad behavior is exactly what a small but growing number of chemists are trying to do. Despite being a chemical language, quorum sensing has long been the purview of microbiologists and geneticists, Janda says. "It's about time more chemists got involved in quorum—sensing studies."

Janda is developing antibodies that could sequester these chemical words and prevent bacteria from chatting with each other. He started with the gram-negative AHLs, which he says are an ideal target for antibody-based anti-infective

therapy, given the highly conserved molecular scaffold and extracellular distribution of AHLs. Next up he's trying to develop antibodies for peptides and AI-2 autoinducers.

At UW Madison, Blackwell has used combinatorial methods to build more than 100 mimics of AHLs. "We chose a combinatorial approach at the outset because we weren't sure exactly which parts of the AHLs were important for quorum sensing. We wanted to sequentially tweak all the parts of the AHL structure systematically to gain new insights and to 'expand' the lexicon," Blackwell says.

Her most promising class of inhibitors features a phenylacetic acyl chain. Substitution on the phenyl ring exquisitely affects activity, with a para-bromo substituent delivering one of the most active inhibitors. Molecular modeling studies show these inhibitors bind the AHL receptor in such a way as to prevent the receptors from adopting an active conformation and subsequently turning on quorum-sensing genes. But more structural studies are required to establish this conclusively, Blackwell says.

Other chemists are developing transition-state inhibitors of bacterial enzymes that build autoinducers. "If you block the synthesis of these quorum-sensing molecules, you block the ability of bacteria to send out these signals in the first place," says [Vern L. Schramm](#), a professor of biochemistry at Albert Einstein College of Medicine in New York City.

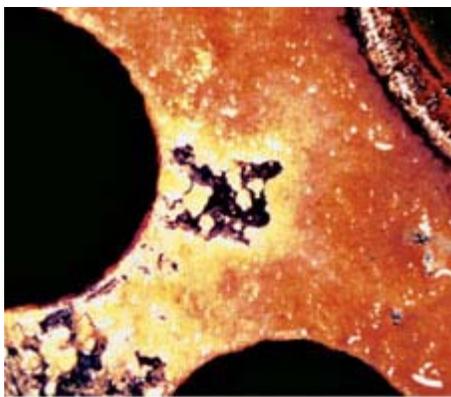
Schramm's group has reported more than 40 transition-state analogs of MTAN, a protein involved in making precursors for gram-negative AHLs and also the generic AI-2 signal. One of his inhibitors binds 91 million times more strongly than the natural substrate.

Despite the potential benefits of blocking bacterial talk, currently only one firm is focusing on quorum-sensing inhibitors for commercial application. Australia's Biosignal is building on a natural class of quorum-sensing inhibitors called furanones, which were discovered in a marine alga called *Delisea pulchra*.



Sarah Everts/C&EN

Opportunity Blackwell (right) and grad student Grant Geske discuss the applications of their quorum-sensing inhibitors.



In the late 1990s, Peter D. Steinberg, a marine ecologist at the University of New South Wales in Sydney, Australia, was curious as to how this seaweed avoids being colonized by bacteria—and even barnacles—in waters rich in microorganisms. Steinberg had identified furanones as the chemicals involved, but he didn't know their mechanism of action. One day he wandered into the office of a colleague, microbiologist [Staffan Kjelleberg](#), and showed him the patent for the furanones. "It was a eureka moment," says Kjelleberg. "When I saw the structures, I said, 'That's a bacterial communication molecule.'" Kjelleberg and Steinberg later showed that *D. pulchra* incorporates a quorum-sensing mimic in its tissue that inhibits bacterial conversations leading to the establishment of biofilms.

[Biosignal](#) was established in 2000 and was publicly listed on Australia's stock exchange in 2004. Although the company has yet to put a product on the market, it is testing whether furanone-coated contact lenses prevent the biofilm formation that causes red eye and irritation. An initial human safety trial on the coated contacts was completed last July, and they will be examined for efficacy next. Biosignal is also developing a suite of paints for boats; the paints prevent barnacle attachment by preventing the formation of bacterial biofilms, which barnacles feed on.

These furanones may have other uses as well. Microbiology professor [Michael C. Givskov](#) at the Technical University of Denmark has shown that algae furanone derivatives block some infections in mice, but the halogenated compounds were initially too toxic to test in humans. Recent structure-function programs have led to nontoxic synthetic furanones that hold promise in initial animal experiments. Novick also has tried to block gram-positive *Staphylococcus* quorum sensing in mice. He found that infections were hampered by the inhibitors, but to date no clinical trials have been done.

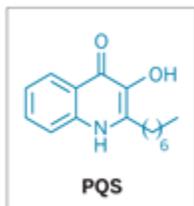


K. Buckingham-Meyer, A. Agostinho, B. Klayman/Center for Biofilm Engineering/Montana State University
Biofilm Babbling Quorum sensing initiates biofilm formation on sink strainers (top), toothbrush bristles

(bottom right), and kitchen pipes (bottom left). The main challenge for clinical applications of quorum-sensing inhibitors may lie in bacteria's verbosity. Researchers don't yet know how much bacteria continue to chat after they've agreed to initiate virulent invasion or form a biofilm. "The real challenge is that by the time someone feels sick, quorum sensing has already happened," Bassler says. "The question is: Does the conversation keep going?"

Either way, you could still use quorum-sensing inhibitors to prevent virulence, says Bassler. "The inhibitors could stop conversations before they happen—for example, before operations, or even in toothpaste," she says.

While some researchers search for quorum-sensing inhibitors, others are searching for new quorum-sensing molecules. A new and unusual autoinducer was recently found in *P. aeruginosa*, which is a linguistic superstar among bacteria. In addition to two AHL autoinducers, this bacterium also chats by using PQS, a quinolone derivative that is so hydrophobic that it has to be trafficked between bacteria through vesicles.



"This was the first case of bacteria using vesicles to communicate," says Marvin Whiteley, an assistant professor of cell and molecular biology at the University of Texas who discovered the vesicle vector. "Many people found this odd, because higher organisms use vesicles to transfer signals all the time, but no one knew bacteria could, too."

PQS also has antibiotic activity against gram-positive competitors in the lungs of cystic fibrosis patients. This is in addition to the bactericidal properties of *P. aeruginosa*'s AHL autoinducer.

Researchers are convinced that many more quorum-sensing molecules remain to be discovered as more bacterial genomes are solved. Bassler, for example, notes that "there are hundreds of different types of microorganisms in teeth. After you brush, biofilms return in exactly the same organization. There has got to be a bigger linguistic tool kit. Currently, we only know a few words. We need to flesh out the entire chemical vocabulary."

Cover Story

- [Bacterial Conversations](#)
- Using a chemical language, bacteria coordinate everything from infection to plaque buildup
- [Battling Biofilms](#)
- It took decades before researchers believed that bacteria were sophisticated enough to organize into slimy biofilms
- [Talking To The Hosts](#)
- Bacteria find ways to have chemical conversations with their hosts as well

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