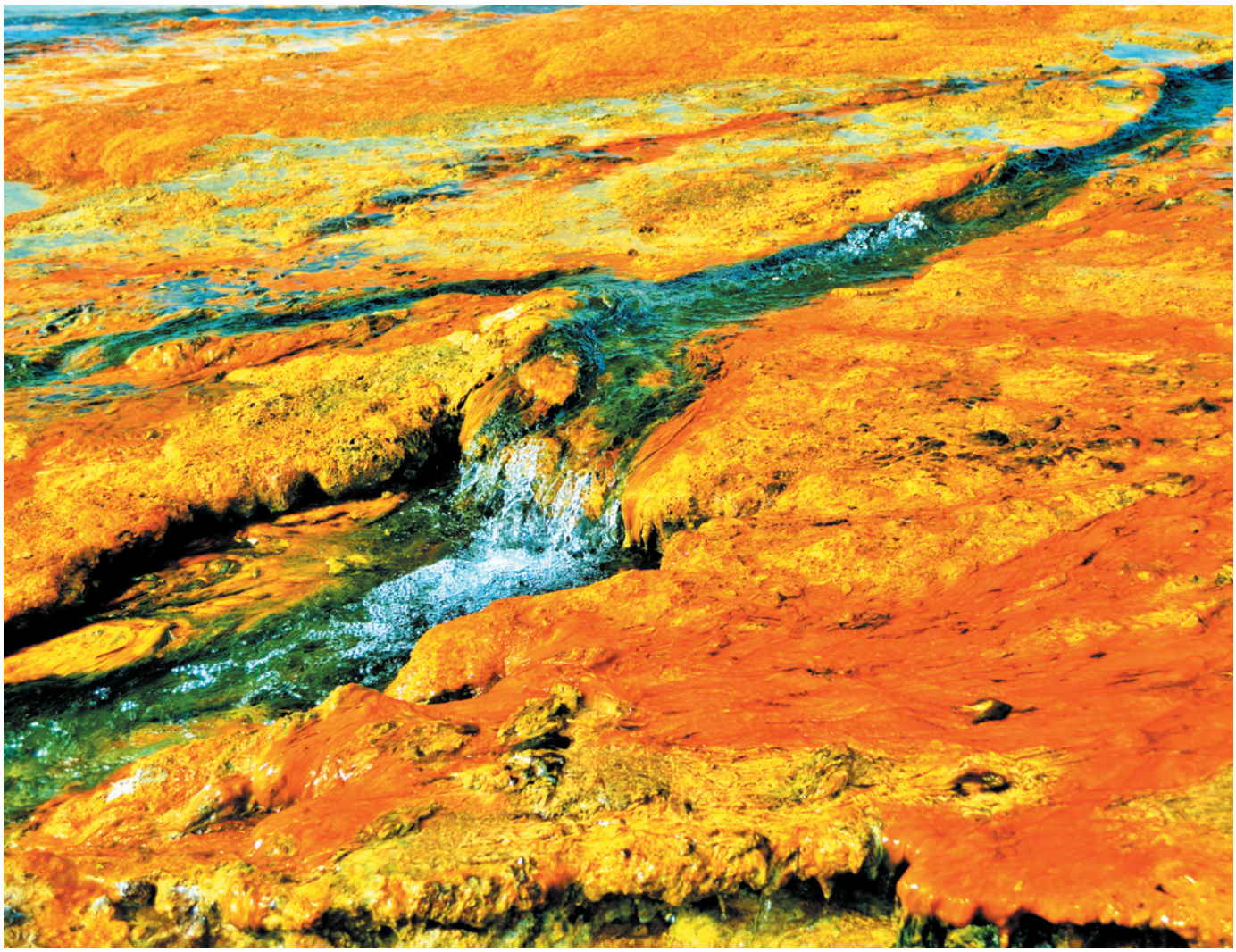
TECHNOLOGY FEATURE

STOP THE MICROBIAL CHATTER

Bacteria can coat everything from thermal springs to teeth. Researchers are looking for antibiotics that can subvert the signalling that the microbes use to carve their niche.

D. G. DAVIES/BINGHAMTON UNIV.



Sheets of communicating bacteria — or biofilms — are a common sight in the run-off channels from hot springs in Yellowstone National Park.

BY VIVIEN MARX

Bacteria are continually evolving ways to avoid the effects of antibiotics, and with the pipeline of new drugs drying up, infections are becoming more and more difficult to fight. As the need for innovative solutions grows, some microbiologists are teaming up with chemists and engineers to try to find ways to subvert the microbes by interfering with the signals they use to communicate.

To undermine the microbes' language, scientists first need to work out what they are saying. Bacteria use chemical signals to synchronize behaviour across a population. That behaviour can help us — in the digestion of food, say — but it can also kill us.

Such molecular coordination is thought to be central to the formation of biofilms — slimy mats of bacteria that spread across surfaces such as hospital catheters or water filtration systems. Some of the bacteria in a biofilm suspend their metabolism, explains microbiologist ▶

Peter Greenberg of the University of Washington in Seattle, making antibiotics less effective because they tend to target bacteria that are still growing. The bacteria can also cover themselves in an armour made of polysaccharides and proteins that antibiotics find difficult to penetrate, says microbiologist Bonnie Bassler of Princeton University in New Jersey.

Such resistance to antibiotics can be treacherous, especially for people who have conditions such as cystic fibrosis that lead to long-term infections. Repeated treatments with broad-spectrum antibiotics heightens the risk that the bacteria will become resistant.

Bacterial communication was first studied in the 1960s, and not long afterwards, researchers found that a marine bacterium known as *Vibrio fischeri* would start to shine brightly once its population reached a certain density[1]. The finding that bacteria will turn their light on synchronously under certain conditions suddenly rendered bacterial behaviour visible and measurable, says Bassler. But because most scientists believed that bacteria were incapable of "fancy things" such as signalling, she says, the collective behaviour was generally dismissed as a "goofy phenomenon of bacteria living in the ocean".

Since then, researchers have observed this 'quorum-sensing' behaviour in many species[2–4] and have started to decipher the biochemistry and genetics of how it happens[5]. They have also been developing devices with which to characterize the messages that are transmitted and received.

In general, quorum sensing is triggered when signalling molecules emitted by individual bacteria pass a certain threshold, at which point the molecules bind to receptors on the bacteria and cause the entire population to express specific genes at the same time. In the case of pathogenic bacteria, the synchronized behaviour can include the release of molecules known as virulence factors, which help bacteria to colonize and harm their host. It also allows bacteria to create biofilms. As the organisms adhere to a surface, they keep signalling to one another. Once they sense a quorum, genes are upregulated and sticky exopolysaccharides are produced that 'glue' the bacteria together.

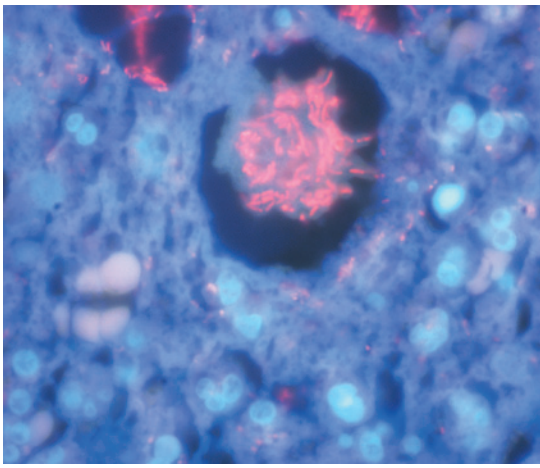
These findings initially led to excitement about the possibility of blocking infection by inhibiting bacterial communication. But the enthusiasm quickly waned when potential drugs failed in early-stage testing.

Now, scientists are taking a more sophisticated approach. The problem with the early work turned out to be in the assumption that the communication required only a few molecules, says Herman Sintim, a chemical biologist at the University of Maryland in College Park. The reality is much more complex, he says. "In human cultures, we all know that it does not take just one word to silence a crowd and so we should not expect that from our distant cousins, bacteria."

It has taken some time, but the research community in this field has grown and researchers have finally amassed enough knowledge about bacterial behaviour to start exploring how to stop the organisms from talking. "We are now getting there," says Bassler. Academics and companies are looking at fresh ways to study bacterial chatter and to create potential communication-disrupting drugs and agents for industrial and agricultural applications.

THE LANGUAGE OF BACTERIA

In developing drug candidates, researchers are sharpening their attack on infections beyond the broad-spectrum antibiotics currently in use. We need to talk to a specific bacterium "in a language only it understands", says Martin Blaser, director of the Human Microbiome Program at New York University Langone Medical Center. Narrow-spectrum antibiotics are less likely to engender resistance because they put fewer species under selection pressure. They also cause less disruption to the body's community of microbes — its microbiome. Broad-spectrum antibiotics will also remain necessary, especially for people who are very ill. In general, they are assumed not to have lingering effects, but Blaser says that "there's more and more evidence that's just not true". They could even wipe out microbial communities involved in the developing metabolism of infants and children.



T. BJARNSHOLT, UNIV. COPENHAGEN

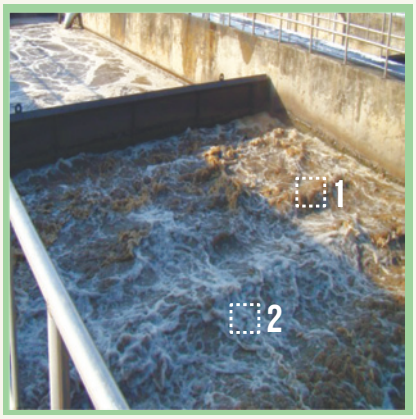
Infection-causing bacteria (red) are often buried deep in tissue and surrounded by white blood cells (blue), making them difficult to target.

It might take some time, but research on bacterial communication will "without question" deliver therapeutic opportunities, says Ronald Farquhar, who directs research at Cubist Pharmaceuticals in Lexington, Massachusetts. Regulatory agencies are particularly open to drug-firm suggestions that will meet the needs of people with chronic infections, he says. For example, someone who needs to use a urinary catheter for a long period of time could take a low-dose agent to stop bacteria from forming a biofilm on the device.

Some drug candidates have already been identified. Microbiologists David Davies and Cláudia Marques from Binghamton University in New York, for example, have found a chemical that some bacteria make to address overcrowding[6]. The bacteria continuously produce *cis*-2-decenoic acid, a communication molecule. When the molecule reaches a critical threshold in a biofilm, a cascade of events is triggered, including changes in gene expression, prompting the bacteria to release themselves from the biofilm and disperse. Davies is now starting a company to commercialize a synthetic

SLUDGE FIGHT

Bacteria can be used to prevent biofilms from clogging the filtration membranes used in wastewater treament.



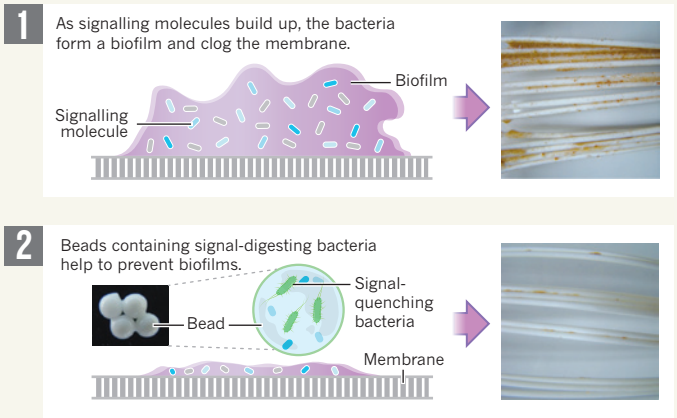


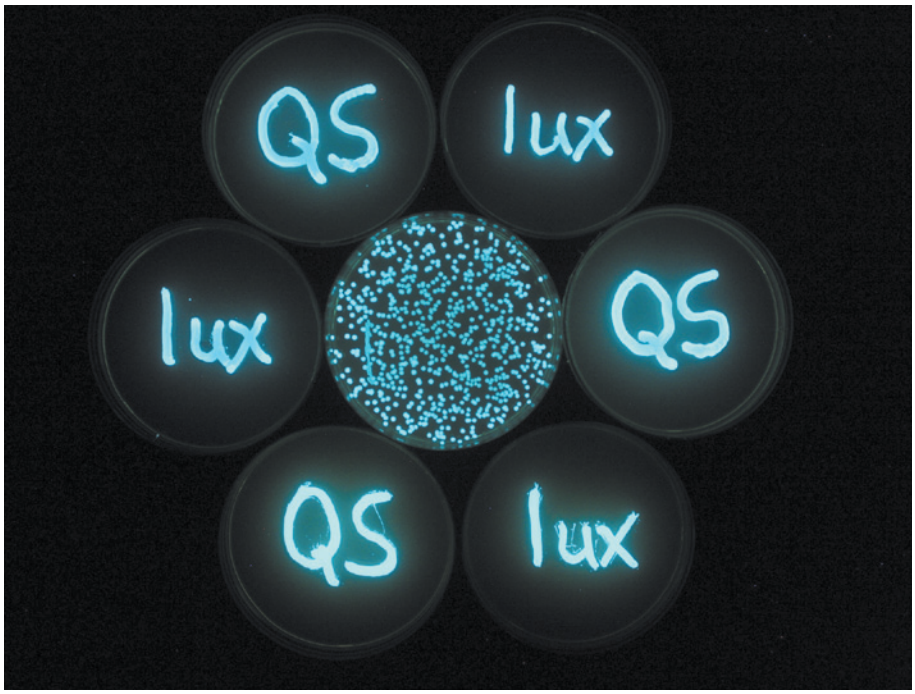
ILLUSTRATION BY CLAIRE WELSH. SOURCE: C.-H. LEE/SEOUL NATL UNIV.

version of the acid for treating acne and disinfecting wounds.

But Greenberg, among others, thinks that caution is in order before moving potential therapies towards the clinic. Dispersing a biofilm could end an infection, he says, but it might also distribute it. "You might be making more trouble than you had to start with," he says.

Indeed, bacterial communication reveals ever more complexities. He has found, for example, that some bacteria in a community are cheats: they do not join the others in secreting enzymes in response to quorum-sensing signals, but still share in the benefits. "There are mixtures of cheats and cooperators in our laboratory experiments," Greenberg says, and a similar mix might be present in the infected lungs of a person with cystic fibrosis. Potential drugs could well be stymied by those cheats. Before developing therapies that disrupt communication, scientists need to know much more about quorum sensing and other bacterial behaviour, he says.

Another complication is crosstalk between species and even across kingdoms. For example, Vanessa Sperandio, who studies bacterial communication at the University of Texas Southwestern in Dallas, has found that the stress hormones adrenaline and noradrenaline, which are present in the gut and elsewhere in the body, can amplify bacterial signalling and increase the virulence of *Escherichia coli* O157:H7 (ref. 7), a pathogen that causes bloody diarrhoea and can be fatal.



A. SCHAEFER, E. P. GREENBERG/UNIV. WASHINGTON

The marine bacterium *Vibrio fischeri* glows brightly when it reaches a certain cell density, or quorum.

OTHER APPLICATIONS

To better understand the complexities of bacterial communication and how to use them against disease, the field is also turning to theoretical work, such as computational modelling and simulation, and to experiments with bacterial pathogens of plants. Greenberg and Lianhui Zhang, at the AStar Institute of Molecular and Cell Biology in Singapore, are working on a project funded by the Chinese government to use quorum-sensing inhibitors on crop pathogens. Such experiments could be proof-of-principle for biomedical applications, Greenberg says.

Quorum-sensing inhibitors could well make it to market in agriculture before biomedicine, says Paul Williams, a chemical biologist and pharmacologist at the University of Nottingham, UK, a hub for bacterial-communication research. Scientists and companies are also testing communication inhibitors for industrial applications. For example, microorganisms are being used in bioreactors to degrade the pollutants in wastewater. The water is then passed through a filter, but a build-up of bacteria can clog the pores of the membrane. The reactor then has to be taken offline, flushed out and cleaned with harsh chemicals such as chlorine — an energy-intensive process that incurs more than half the cost of running a membrane bioreactor, says Chung-Hak Lee, a chemical engineer at Seoul National University.

> *"There are mixtures of cheats and cooperators in our laboratory experiments."*

Lee has come up with a potential solution. His approach taps into a typical communication network found in biofilms, in which enzymes secreted by some species digest signalling molecules emitted by others. He and his team isolated such signal-quenching bacteria and placed them in beads that contain pores that keep the bacteria in, but let signalling molecules pass through. When placed near the filtration membrane in a bioreactor, the beads undermine bacterial communication and help to stop biofilms from forming (see 'Sludge fight'). In lab tests and in a pilot-scale wastewater treatment plant, Lee has found that the beads save almost half of the energy costs of a conventional membrane bioreactor.

Several companies are exploring how to prevent biofilms for industrial and biomedical applications. Selenium, a spin-off company from Texas Tech University in Austin that is backed by the venture-capital firm Emergent Technologies, is developing selenium-containing coatings that could protect materials such as catheters, contact lenses and voice prostheses by producing reactive oxygen molecules that ward off bacteria.

Another company, Curza, founded last year in Salt Lake City, Utah, is developing coatings that prevent biofilms from forming on hip and knee implants. Its research involves chemical synthesis, molecular genetics, mass spectrometry and scanning electron microscopy, as well as a proprietary flow cell assay that better represents physiological conditions by using liquid flow rather than stagnant broth assays. The company says that the assay can help to characterize whether a biofilm is prevented under real-life-like conditions and show what might happen as an antimicrobial compound dilutes away from a medical device's coating, for example.

And Kane Biotech of Winnipeg in Canada is developing combinations of antimicrobials and biofilm inhibitors for coating biomedical devices, treating wounds and protecting teeth and skin. Sri Madhyastha, chief scientific officer, says that one of their products has been licensed by a medical-device company. Kane also sells products through veterinarians and distributors, including a water additive aimed at preventing plaque from forming on the teeth of pets.

The company tried to obtain approval from the US Food and Drug Administration for an anti-biofilm enzyme in a wound-care product, but as a new chemical entity, it would require extensive testing. That route "is too expensive and time-consuming", Madhyastha says, so the company has put this product on the back-burner.

OBSERVATION PLATFORMS

To test their potential products, Kane's researchers use confocal microscopy and an instrument called the CDC Biofilm Reactor: a 1-litre beaker containing 8 slim rods around which liquid moves. Dotting the length of the rods are 24 circular disks on which biofilms can be grown and tested. The reactor was built under a licence from the US Centers for Disease Control and Prevention by BioSurface Technologies of Bozeman, Montana, which sells several other types of vessel in which scientists can grow and disrupt biofilms in a controlled, standardized environment.

UNIV. COPENHAGEN

Thomas Bjarnsholt wants assays that mimic the way that bacteria can be shielded from antibiotics.

At Fluxion Biosciences in South San Francisco, California, cell biologist Bryan Haines helps labs to set up the firm's BioFlux microfluidic platforms. The platforms allow scientists to do 24 biofilm experiments on one multiple-well plate. The temperature and gas content in the medium can be adjusted to suit the preferred growth conditions of the bacterium being studied. The wells are the reservoirs for reagents, potential antibiotics and bacteria; running underneath them are micrometre-scale channels in which a biofilm can grow. The plate is sealed at the top and users select the pressure with which to distribute fluids and cells through the channels, then observe the biofilm through an inverted microscope.

But Sintim says that scientists need better assays if they are to study the subtleties of bacterial communication. Cells live in a three-dimensional architecture and respond to many cues. And biofilms contain multiple species, making a specific biofilm hard to culture using traditional approaches. "Many systems that have been developed to date are reductionist systems," Sintim says, "and it is not obvious to me if data obtained from these reductionist platforms have any biological meaning."

Together with bioengineer William Bentley at his university, Sintim is developing a microfluidic system that will not just track cells moving through a three-dimensional space, but will also let experimenters perturb conditions and measure changes in appearance and behaviour. Their system uses a membrane to separate two types of bacteria. On one side of the membrane are bacteria they have engineered to fluoresce green under ultraviolet light. These bacteria secrete signalling molecules that can pass through the membrane. On the other side are bacteria engineered to fluoresce red only when they receive that signal. The device allows researchers to alter the environment of each side independently and to control the rates of flow of liquids across the device (see 'Just watch'). Scientists can then study the effect of different gradients in a setting that is more typical of, for example, the body.

Thomas Bjarnsholt helps university-hospital physicians to diagnose infections and has a microbiology lab at the University of Copenhagen, where he is building a system for studying biofilms. Current assays do a poor job of showing how slowly a biofilm forms on a medical implant, he says, so he wants to develop an assay that more closely mimics the *in vivo* conditions. Also, only a few people develop infections when their hips or knees are replaced, so he hopes to determine what makes some luckier than others.

In his view, a communication disrupter should be tested not just by adding it to a biofilm. In the chronically infected lung of a person with cystic fibrosis, antibiotics have to travel through the bloodstream, then diffuse through necrotic material, mucus and pus to get to the infection site. "It's all embedded in slime," he says. The slime also has anaerobic pockets, where antibiotics tend to fail. Just 40 micrometres of pus or mucus suffice to create such pockets. He is developing surfaces, gels and other media that mimic this kind of shielding and allow researchers to take this into account.

Quorum-sensing inhibitors and other communication disrupters will eventually emerge, Bjarnsholt predicts[8]. An area of interest for him is dressings, especially for people with diabetes, who repeatedly develop wounds. At the moment, dressings often contain silver, which acts as an antibacterial treatment, but infections still develop, so new approaches are needed, he says.

But new antibiotics will need more-expensive tests that require greater expertise to administer, says Sperandio. The standard way to test antibiotics is the minimal inhibitory concentration test, which measures the concentration at which a compound needs to be administered to stop bacteria from growing. Williams points out that this approach "is obviously of no use" for assessing compounds that disrupt communication.

In fact, says Sperandio, the whole communications approach to curing infection is at odds with the long-held dogma that a cure means killing the microbes. Communication disruptors could prevent pathogenesis without killing the pathogen, for example. Except in rare cases, such as infections of heart valves, it is not necessary to kill every bacterium, says Blaser. Even conventional antibiotics do not sterilize an organ; they reduce replication rates and "ultimately it is the immune response in patients that clears the infection", he says. New antibiotics could battle bacteria in this way, too.

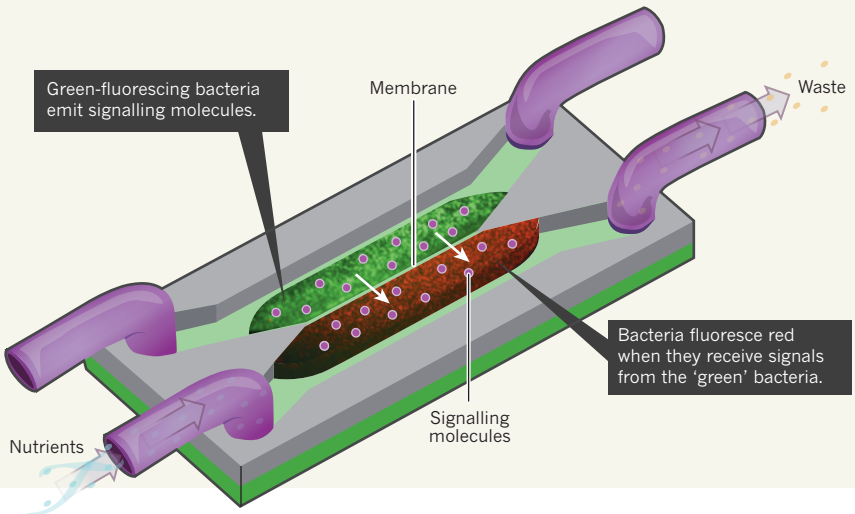
The war on harmful bacteria is most definitely a war that humans need to win, says Blaser. But that does not mean we have to harm ourselves in the process."We don't want a Pyrrhic victory," he says. ■

Vivien Marx *is technology editor for* Nature *and* Nature Methods.

ILLUSTRATION BY CLAIRE WELSH. SOURCE: W. BENTLEY, G. RUBLOFF, X. LUO, H. SINTIM/UNIV. MARYLAND

JUST WATCH

Researchers at the University of Maryland are building a microfluidic device to study bacterial signalling. A membrane separates two types of bacteria — one fluoresces green and the other red — but allows the passage of signalling molecules. The device allows scientists to change the flow rate of liquids to look at concentration gradients, as well as to adjust various environmental factors, then observe how that affects communication between the bacteria.



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