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Researchers turn to tiny robots to fight antibiotic resistance

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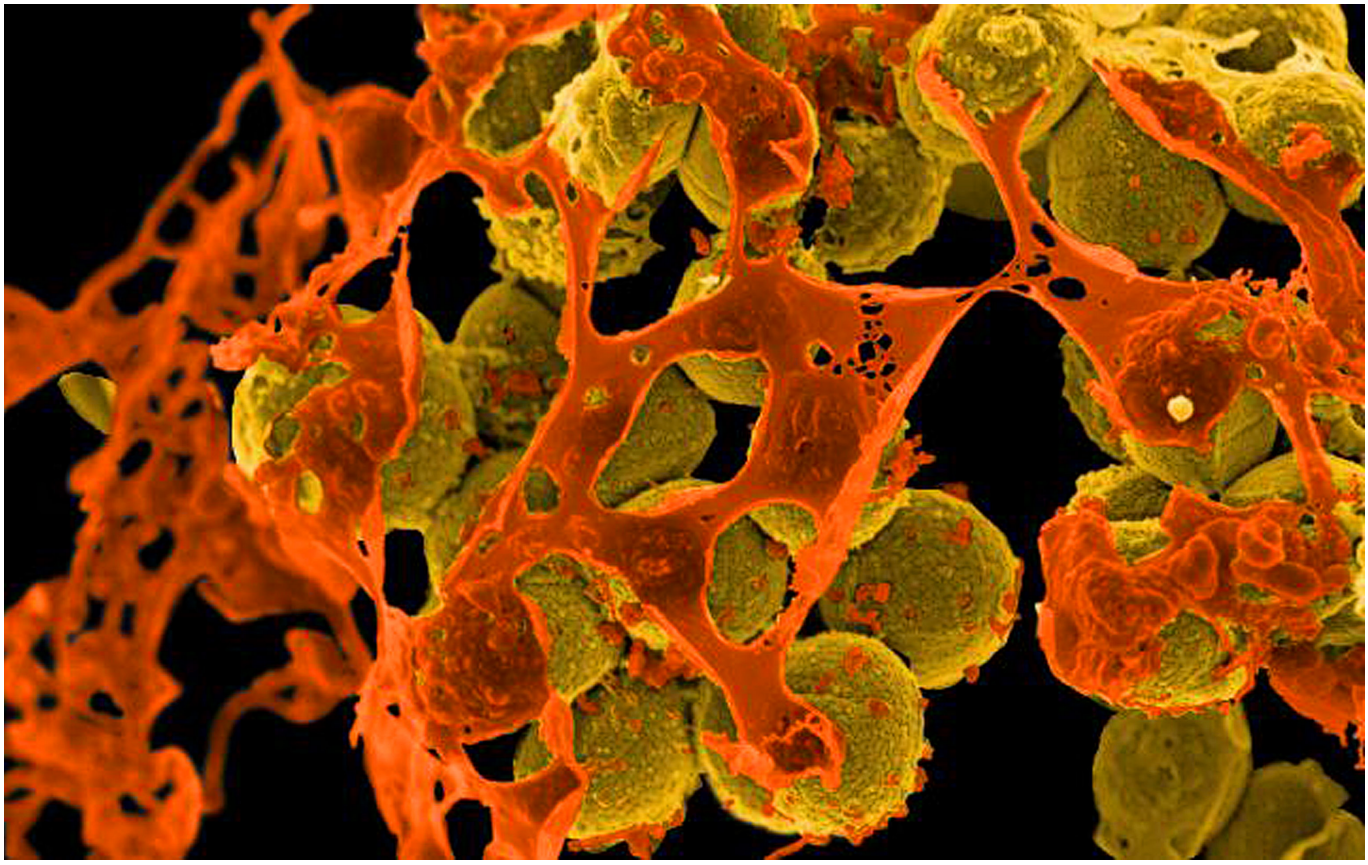

Nanomachines could some day offer a novel approach for treating dangerous infections from MRSA, shown here in a digitally colorized, scanning electron microscopic image in which orange-colored cellular debris surround the mustard-colored spherical bacteria. Image credit: National Institute of Allergy and Infectious Diseases.

Antimicrobial resistance remains an urgent problem, yet new drugs are devilishly hard to find, develop, and test. And even successful breakthroughs are only a temporary fix. "Bacteria have developed resistance to all traditional antibiotics," says microbiologist Ana Santos at Rice University in Houston, TX, and at Fundación Instituto de Investigación Sanitaria Islas Baleares in Palma, Spain. "We need to try something completely different that they don't already know from history and haven't been exposed to throughout their evolution."

Santos has been collaborating at Rice with chemist James Tour on such a prospect. Rather than searching for new antimicrobial compounds, their group uses what might best be described as a brute-force approach. The researchers have recently been designing tiny, spinning molecular machines, which travel to the site of an infection and drill holes in infectious pathogens, tearing the tough outer membranes apart. Without the outer membrane, the vulnerable innards spill out, and the cell dies. In lab tests, the molecular machines can puncture a wide variety of pathogens, acting as a sort of synthetic antibiotic that can even effectively kill bacterial populations resistant to antibiotics and persisters, a subpopulation of cells thought to promote resistance (1).

Such collaborative approaches between microbiologists and chemists offer a fundamentally new way to fight disease. Where antibiotics take a biological approach, nanomachines offer a decidedly mechanical one. "It's really taking a tool from the chemistry realm and applying it to biology," Santos says. Recent experiments by Santos and Tour—and other groups—have tested nanomachines against cell lines and in animal models of antimicrobial-resistant infections. Early findings have been promising, hinting at a range of biomedical applications. Whether they

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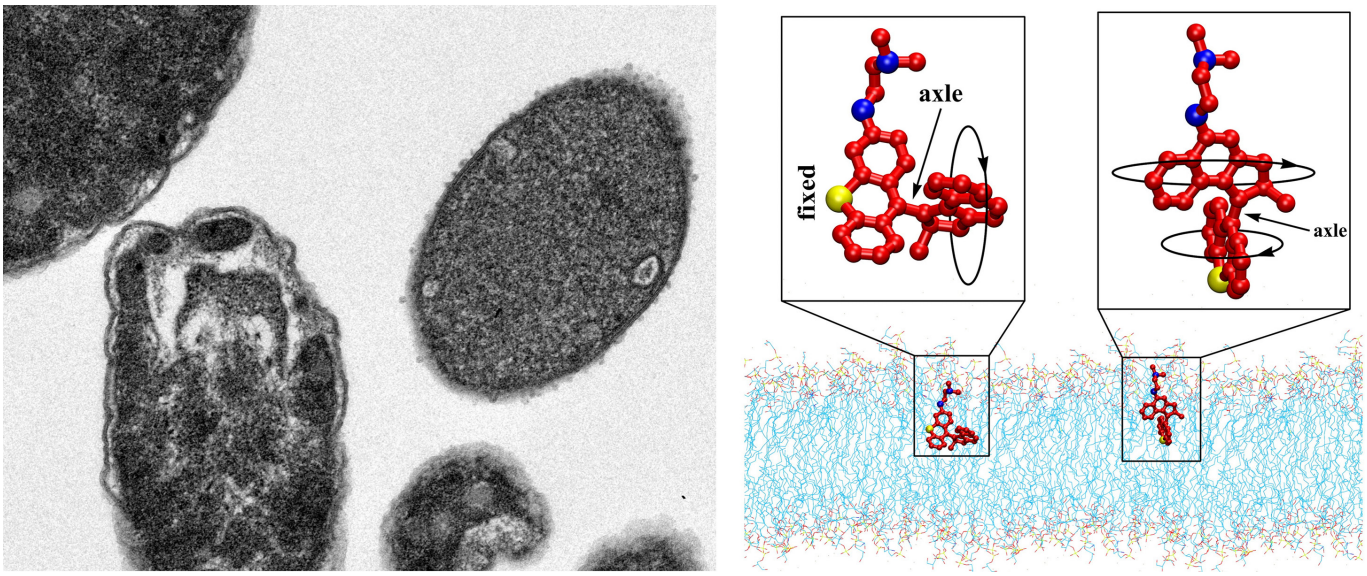

Light-activated molecular machines (schematic of two variants; *Right*) drill into and destroy antibiotic-resistant bacteria. Experiments with *E. coli* (*Left*; transmission electron microscope image) suggest that the bacteria degrade after exposure to light-activated molecular drills. Image credits: (*Left*) Matthew Meyer (Rice University, Houston, TX). (*Right*) Tour Research Group (Rice University).

can be successfully translated into real-world, clinical settings, however, remains to be seen.

Small Solutions to a Big Problem

Nanomachines, or artificial molecular motors, refer to synthesized devices that typically measure less than a micrometer in size and can be controlled to complete a task. Over the last decade or so, researchers have designed minuscule contraptions that incorporate rotors, shuttles, muscle-like components, and other moving parts (2–4). Pioneering chemists and engineers have even synthesized tiny cars, elevators, and pumps made from a cluster of atoms. Some, including nanomachines developed by Tour at Rice, are powered by light; others get energy from chemical reactions or electrical energy. In 2005, Tour led the design of the world's first vehicle at the nanoscale, a two-wheeled miniature marvel made from a single molecule. It was a little wider than a molecule of DNA. Its spinning wheels were made from buckyballs—60 atoms of pure carbon tightly arranged to make a hollow ball—and organic groups made up its chassis and axle (5). In 2006, Tour modified the nano vehicle to incorporate a motor based on a design by organic chemist Ben Feringa. Molecular machines even earned Feringa and others the 2016 Nobel Prize in Chemistry. But thus far, these mini bots have been a promising technology without an obvious application. Some think antibiotic resistance might fit the bill.

Already, Tour and many others have started exploring their use in medical applications. In 2017, he and his colleagues manipulated the machines to attach to living cells and, when activated by ultraviolet (UV) light, burrow into the thick lipid bilayer on the outside of the membrane (6). In lab experiments, the molecular machines effectively killed cancer cells in a way completely unlike available therapies.

"It's a whole new domain in treatment," Tour says. Using molecular machines, he says, is like conducting surgery with a scalpel at the cellular level: "This is a new modality where you have a mechanical action at the nanometer scale." It's hard to imagine how a cancer cell could develop defenses. "Can a tumor build resistance to a scalpel?" Tour asks.

That 2017 proof-of-concept caught the attention of Santos, whose work focused on antibiotic resistance. "I thought, this is amazing; this is something that could work for pathogens," she says. "These were mechanical drilling mechanisms that do not exist in nature."

It was an important first step, but there was a hurdle: Tour had activated the machines with high-energy UV light that, in living systems, could damage surrounding healthy tissue. And it would be difficult to get a UV light source into the body with the machine. But when Santos contacted Tour to learn more, she learned that he was working on a less destructive energy source—visible light. Tour suspected that by adjusting the makeup of the machines, such as by adding nitrogen atoms, they could create devices that could harvest enough energy from the visible spectrum to kill cells.

Santos, a microbiologist, had spent years investigating the biological damage that UV radiation inflicted on bacteria, especially pathogenic strains resistant to antibiotics. So when she heard Tour's plans for modifying the machines, she immediately saw a way to combine their interests. Maybe, she thought, the cell-destroying machines could be harnessed against bacteria.

Bacteria vs. Machines

The two spent the next year unpacking the complexities of pathogenic cells. "Ana was constantly teaching me how rapidly they reproduce, change, and share DNA with each other," he says. "They're really sophisticated little things." The researchers modified his original designs and began running calculations of how much energy they'd need to bore through bacterial membranes. "We had to modify the design of the molecule extensively, and then try to understand why this thing was working," Tour says.

Tour came to realize just how big a challenge was posed by bacterial cells, compared to other cells he'd tested in the

past. "We were blissfully unaware of how tough the cell wall really is," he says.

Finally, by 2021, they had settled on a range of nanomachine designs that, because of the added nitrogen, could likely spin fast enough to bore into cells when activated by visible light. This new generation of machines runs on light at 405 nanometers, the violet-blue end of the visible spectrum. And in their latest report, published in *Science Advances* last June (7), the group described what happened when they set molecular machines on a variety of Gram-positive and Gram-negative bacteria. Gram-negative bacteria have thin cell walls surrounded by a thick membrane, whereas Gram-positive bacteria have a thick cell wall and no outer membrane. The test subjects included strains of Gram-negative *Escherichia coli* and *Acinetobacter baumannii*, as well as methicillin-resistant *Staphylococcus aureus* (MRSA), Gram-positive bacteria that live on the skin and can cause staph infections.

> **"We've combined principles from nanotechnology with antimicrobial design."**
>
> **— Cesar de la Fuente-Nunez**

The experiment began with 19 molecular machine configurations that differed based on where the researchers had spliced in molecular groups (amines, for example, brought nitrogen atoms). Some had additional groups in the rotors; others had them in the "body" of the machine. The researchers suspected that the additional groups would help the machines harvest energy from visible light. But they needed experiments to determine where exactly to add those groups.

After testing the designs on *E. coli* cultures, the researchers winnowed the field down to six. (They found, for example, that slower-spinning machines didn't burrow as deeply into the bacterial membrane and, hence, were less likely to inhibit the growth of *E. coli*.) In the expanded tests on multiple strains, the best-performing molecular machines killed the bacterial cells in as little as 2 minutes.

When the researchers investigated the action using RNA sequencing and advanced microscopy, they found that the successful machines had disrupted the bacterial membrane enough for material within the cell to flow out. In a follow-up experiment, they found that the puncture wound made by less powerful machines could still be useful: When they combined common antibiotics with these molecular machines, they were able to kill even stubbornly resistant strains in a matter of minutes. They also found that the molecular machines successfully eliminated the persister cells that are thought to help bacteria gain resistance.

Even after repeated exposures, the treated cell lines didn't show any signs of developing resistance to the synthetic antibiotic, Santos says. Resistance occurs when resistant genes evolve or bacteria acquire them from other bacteria. Santos doesn't foresee new genes stopping a mechanical, molecular drill that's spinning at millions of times per second.

Already, they've tested the approach on wax moth larvae, whose similarities with the mammalian immune system make them a common model for infectious disease. The researchers inflicted the larvae with burn wounds and infected them with one of the two bacterial pathogens, *A. baumannii* and *S. aureus*. All untreated larvae died within a week of treatment, whereas treatment with nanomachines extended survival beyond a week for 25–60 percent of the animals.

Indeed, a better therapy for MRSA could be a boon for the treatment of burn victims. About three-quarters of all deaths from patients with severe burns arise from systemic bacterial infections that begin in the wound as MRSA or some other infection; some studies estimate that half of all burn victims in hospital intensive care units will have an MRSA infection (8). Because burn wounds are on the skin, Santos says, molecular machines would have plenty of visible light available.

But Tour envisions going deeper. "Right below the surface of the skin is easily accessible with visible light. Or the oral cavity, colon, and rectum," he says. "There are lots of areas where you can easily get light without having to pierce the body."

A Challenging Domain

Tour and Santos are not the only ones who see promise in treating resistant infections with molecular machines. At the February 2022 American Association for the Advancement of Science meeting, the winning entry for a student e-poster competition described lab experiments that tested various concentrations of fast-spinning molecular machines—like the ones developed in Tour's lab—on large viruses and at varying durations of exposure to visible light (9). The best-performing concentration reduced the viral viability by 97%, and the research suggests that molecular motors could one day target viruses as well as bacteria (9).

A strength of these approaches could be their precision, according to recent work. Antibiotics are usually administered to an entire system, hence speeding up the selective pressures that spur drug resistance. Excessive use further increases the chances of resistance. A nanomachine approach would sidestep the pitfalls of antibiotic overuse, says microbiologist Cesar de la Fuente-Nunez at the University of Pennsylvania, in Philadelphia. "It can move through the wound and deliver the therapeutics directly to the infection site," de la Fuente says. Together with chemist Samuel Sánchez at the Institute for Bioengineering of Catalonia in Barcelona, Spain, de la Fuente is using nanomachines as a kind of precision shuttle, but for infected wounds. "We've combined principles from nanotechnology with antimicrobial design," he says. They've shown that nanomachines can carry an antibiotic payload directly to a pathogen known to be vulnerable (10).

Their silica-based machines can carry a payload of a potent antibiotic derived from wasp venom. The machine gets energy from an enzymatic reaction involving urease, which is laid out in a liquid trail by the researchers. It follows this trail to the infection site. (In practice, such a trail might lead the machine across the skin and to an infected wound.) The machines included hollow silica balls, filled with the antibiotic, which were capable of self-propulsion driven by the activity of urease. The researchers tested their designs on mice with skin abscesses infected with *A. baumannii* and found that the tiny machines could reduce the bacterial load.

While the technology naturally lends itself to skin infections, de la Fuente notes that recent results suggest a nanomachine could, in principle, deliver other kinds of payload—even chemotherapy or immunotherapy, for example. Thus far, however, progress has been limited to tests on cell lines and animal models.

To pave a path to practical applications beyond skin, researchers will have to ensure, among other things, that the machines have an accurate targeting mechanism that allows them to move deeper into the body without harming living tissue. If they were to be used, say, for preventing cancer deaths—the vast majority of which arise from metastases or cancer cells in the body—researchers would need to both train the devices to recognize cancer cells and develop a power source that could safely function in the bloodstream. The goal, whether for cancer or infections or other applications, de la Fuente says, would be to create something that could be easily ingested, readily digested, and safely cleared after it did its job.

Tour is now testing the limits of molecular machines powered by infrared light, which has less energy than visible light, but may be easier to transmit. He's also investigating whether molecular machines might be used to kill fat cells—an application that, he says, would be "big business." In de la Fuente's lab, they're looking for ways to load other small molecules on their tiny devices. He also has his sights set on a model of treating systemic sepsis; the idea is that the molecular machines would carry powerful antibiotics to the infection that set off the reaction. Santos wants to expand testing to pathogenic fungi and even parasites like the ones that cause malaria. But she remains intensely focused on antibiotic resistance. Pathogen-drilling molecular machines may still be years from clinical utility, she acknowledges, but conventional approaches are faltering. Says Santos, "We really do need to think outside the box."

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