and require high voltages. Key advances in the current work are the optimization of a mechanical transmission to generate the appropriate force–displacement characteristics and the development of a lightweight electronic circuit that converts the low voltages generated by the solar panels into the 200-volt pulses needed to power the piezoelectrics.

All these components are combined to produce the resulting test system — a tall, gangly device, which has its solar panels perched high above the wing system and its electronics hanging below. It is certainly not the most aesthetically pleasing flyer, but when the lights come on, it lifts off and achieves sustained, autonomous, untethered flight. Although the device by itself is an impressive achievement, equally rewarding is the detailed description of the modelling and design that the team has put into the system. The flight of the RoboBee represents much more than just the sum of the parts. It also reflects the successful compromise that has been achieved between the competing interests of weight, power, control, strength, resilience and even cost.

There is still much work to be done, and we are not quite at the point at which a robot swarm will take to the skies — as is nightmarishly depicted in dystopian science fiction such as Michael Crichton's novel *Prey*. Jafferis and colleagues' robot requires intense light to generate sufficient power for take-off (at least three times the intensity of the Sun). Moreover, the robot flies for just under a second before veering off out of view, presumably heading for a crash landing. Nevertheless, advances in battery technologies could soon eliminate the need for solar panels, and with the ever-improving capabilities of small-scale electronics and communication technology, the controlled flight of tiny robots seems within our grasp. ■

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ANTIBIOTICS

Death from within

Some bacteria naturally transfer pieces of their DNA within and between species. Such a piece of DNA has been engineered to act as a molecular 'Trojan horse' that unleashes a toxin to selectively kill antibiotic-resistant *Vibrio cholerae* bacteria.

SANNA KOSKINIEMI & PETRA VIRTANEN

Antibiotic resistance among infectious bacteria is an increasing problem worldwide, resulting in large part from the overuse of antibiotics. Writing in *Nature Biotechnology*, López-Igual *et al.*[1] demonstrate a nifty way to selectively poison antibiotic-resistant *Vibrio cholerae* bacteria — the species that causes cholera — from the inside. The authors' aim is to offer a highly targeted alternative to standard broad-brush antibiotics.

Our present scattergun overuse of antibiotics has caused several problems, one being the emergence of antibiotic-resistant bacteria. Another is that typical broad-spectrum antibiotics affect not only the target disease-causing (pathogenic) bacteria but also our normal beneficial bacteria, which protect us against infection and might influence many other aspects in humans, including weight, mood and allergies (see go.nature.com/31x0csa). The specificity of the approach proposed by López-Igual and colleagues could avoid both of these issues.

The authors' method builds on the ability of bacteria to transfer certain pieces of genetic material ('mobilizable' DNA) on cell-to-cell contact, in a process known as conjugation. López-Igual *et al.* take advantage of this phenomenon to transfer a set of genes that encode a toxin (a protein called CcdB) and its antidote (a protein known as CcdA) from donor bacteria into their neighbours. The system is designed so that the toxin will be made only in *V. cholerae* and the antidote will be made only in the *V. cholerae* bacteria that are antibiotic-sensitive, so that just antibiotic-resistant *V. cholerae* will be killed (Fig. 1).

López-Igual *et al.* used several clever tricks to ensure that toxicity occurred only in the target cells. First, they engineered the toxin-encoding genes to be under the control of a *Vibrio*-specific protein, the transcription factor ToxR (which is essential for *V. cholerae* to cause

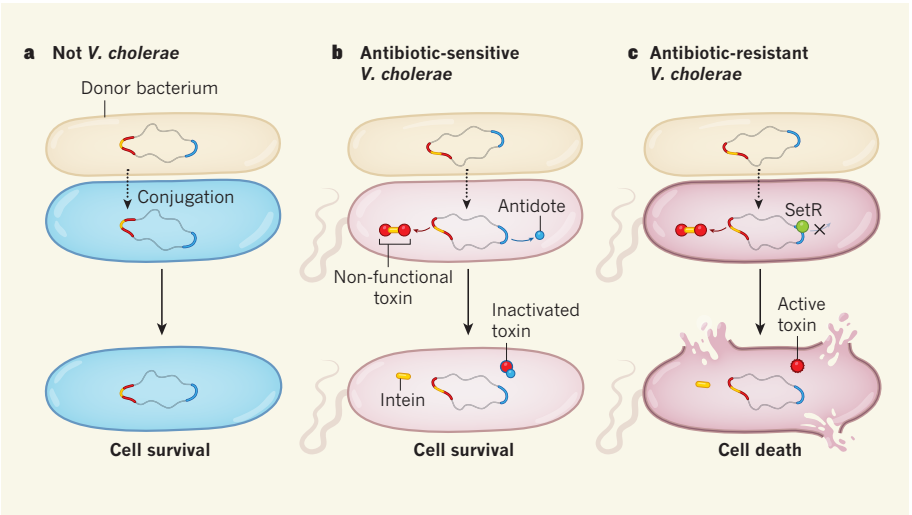

Figure 1 | Time-delayed destruction of *Vibrio cholerae*. López-Igual *et al.*[1] have designed a system with which to selectively kill antibiotic-resistant *V. cholerae*, the bacterial species that causes cholera. They engineered a circular piece of genetic material that encodes both a toxin (red) that is interrupted by a component known as an intein (yellow) and an antidote to the toxin (blue). These genes are inserted into a donor bacterium, and can then be transferred into other bacteria in a population through a transfer process called conjugation (dotted arrows). **a**, If the bacterium that receives the genetic material is not *V. cholerae*, then the toxin and antidote are not expressed because the bacterium lacks the appropriate transcription-factor protein that drives their expression. Such cells survive. **b**, If the recipient is antibiotic-sensitive *V. cholerae*, the bacterium makes the antidote and a toxin that is non-functional because it contains an intein. Over time, the intein removes itself from the toxin. However, the resulting functional toxin is inactivated by the antidote, and the bacterium lives. **c**, If the recipient is an antibiotic-resistant *V. cholerae*, expression of the antidote is blocked by a repressor protein called SetR, which is encoded by a gene that contributes to antibiotic resistance. When the intein removes itself from the toxin, this generates active toxin and the bacterium dies.

disease). This means that, if the toxin-encoding genes were ever transferred by conjugation to other bacterial species, no toxin could be made. Second, to ensure that any antibiotic-sensitive *V. cholerae* recipients do not die, the authors included the gene that encodes the antidote on the mobilizable gene set. This antidote is made in all antibiotic-sensitive *V. cholerae* but not in antibiotic-resistant *V. cholerae*, because in the latter bacteria, antidote production is turned off by a repressor protein, SetR, which is encoded by, and also regulates, the antibiotic-resistance genetic element *SXT* in these bacteria.

However, simply having the antidote present might not be enough to prevent the killing of bacteria that are not intended to be targeted. The CcdB toxin is highly potent and acts rapidly, killing bacteria by causing extensive damage to their genetic material — it acts by inhibiting an enzyme called gyrase, locking it to DNA, which results in DNA breaks. The authors therefore built in a delay mechanism — generating a ticking time-bomb that becomes deadly only after some time.

To do this, they inserted a genetic module that encodes a special protein known as an intein into the toxin gene. Expression of the modified toxin gene produces a non-functional toxin, from which the intein protein excises itself over time through a process called splicing, thereby generating the functional toxin. The effect is to delay the deadly action of the toxin, allowing time for bacteria that receive it to respond. The time it takes for the toxin to mature allows a bacterium that is antibiotic-sensitive to produce enough antidote to survive. If the bacterium is antibiotic-resistant, however, no antidote is produced and, after the toxin has matured, the cell will die.

López-Igual *et al.* went on to show that their approach is not limited to a single type of toxin protein, but that others — namely, HigB2 (which targets a type of enzyme called an mRNase), RelE4 (which inhibits protein synthesis) and ParE2 (another gyrase inhibitor) — are also functional after intein-mediated splicing. Finally, they tested their method in three natural *V. cholerae* habitats: water, zebrafish larvae and crustacean larvae. They found that their approach could eradicate antibiotic-resistant *V. cholerae* in all three habitats. The specific regulators they used work only for *V. cholerae*, but the system could easily be adapted to target different bacteria.

Other studies[2,3] have used bacterium-infecting viruses as well as conjugation methods to deliver DNA- or RNA-digesting enzymes called nucleases to target drug-resistant bacteria. How does López-Igual and colleagues' approach compare with these? One advantage of their system is that fewer bacteria that can resist the internal threat evolve, with around one such 'escape mutant' per 10^7 bacteria that receive the toxin, which is a level of escape mutants that is least a hundred times lower than is found with a virus-based approach[2,3].

Nevertheless, this escape rate is not low enough to prevent the development of resistance. One factor to consider is the typical population sizes of the bacteria being targeted[4]. If the population is larger than the size predicted to generate an escape mutant, then resistant bacteria will already be present. People who have cholera produce around 10^8 *V. cholerae* bacteria per gram of faecal material, and water reservoirs associated with an outbreak of the disease would probably host an even higher number of bacteria[5]. In such large populations, it is probable that thousands of mutant cells would be resistant to the toxin, and would be unaffected by the killing system described here. Further research should attempt to work out the mechanisms behind this resistance, and to find ways to optimize the system.

"The authors' aim is to offer a highly targeted alternative to standard broad-brush antibiotics."

A problem with conjugation approaches in general is that they are inefficient, with only a few cells out of a hundred actually receiving the genes. Two things will probably be necessary to generate a functional therapy: first, the use of several toxins and/or delivery systems, to try to limit the number of escape mutants; and second, improvements in the efficiency of gene transfer. Although López-Igual and colleagues' system will probably not immediately solve the problem of antibiotic-resistant cholera infections, it might make an important contribution to the arsenal of alternative treatments for critically ill people.

One final question is perhaps more profound. The severe watery diarrhoea that is characteristic of cholera causes an estimated 100,000 deaths every year[6]. With this grim statistic in mind, why target just the antibiotic-resistant bacteria when you could try to kill them all instead? A possible benefit could be a lower selection pressure on *V. cholerae* to become resistant to this new killing method. However, one would need to know in advance that the infection is indeed antibiotic-resistant, or it might be necessary also to use antibiotics in parallel to treat the infection. Perhaps the system described by López-Igual *et al.* should be viewed as an intriguing proof-of-concept of how selective antibiotic alternatives could be used in the future: it could easily be modified to target all *V. cholerae*, whether or not they are antibiotic-resistant. ■

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PHYSICAL CHEMISTRY

Crystallization tracked atom by atom

Atoms of a metal alloy have been tracked as they form crystal nuclei — the first ordered clusters of atoms or molecules produced during crystallization. The findings might help to develop a general nucleation theory. SEE LETTER P.500

PETER G. VEKILOV

Nucleation is the earliest stage of crystallization, in which atoms or molecules dispersed in a crystallization medium first come together to form ordered clusters known as nuclei. Crystal nucleation underpins a vast range of phenomena, from the solidification of rocks from molten magma to the hardening of biological tissues through the formation of various minerals, and the protein fibrillation and crystallization associated with a plethora of diseases. In many instances, nucleus formation represents the rate-limiting stage of crystallization and determines the main properties of a crystal population, including the type, number and size distribution of the crystals that form. On page 500, Zhou *et al.*[1] report features of crystal nucleation that not only clash with several assumptions of classical nucleation theory, but also go beyond more recent non-classical models.

Crystal nucleation occurs in a medium (a solution, melt or vapour) that is supersaturated with respect to a crystal. In other words, the concentration of the material dissolved in