Much of the success of Vesuna and colleagues' study relies on the reversible dissociative effects of ketamine. At subanaesthetic doses, this fascinating drug elicits dissociation and pain relief (analgesia), and has antidepressant and anti-suicidal properties. At these doses, electroencephalograms (EEGs, which detect neuronal activity at the surface of the brain) show that ketamine broadly dampens 8–12-Hz oscillations[3]. At higher doses that induce unconsciousness, EEGs reveal a rhythm in the brain's frontal lobe in humans that alternates between low (1–4 Hz) and high (27–40 Hz) frequencies[4]. Given that these changes occur over large areas of the brain's surface, it is striking that a small layer of deep cells is specifically responsible for dissociation. To our knowledge, the oscillations described by Vesuna *et al.* have not been reported previously for ketamine. This is probably because surface EEG recordings cannot detect localized rhythms generated deep in the cortex.

Rapid technological advances are producing increasingly sophisticated techniques to manipulate neural circuits with precision and high temporal resolution. Vesuna and colleagues' work exemplifies how these advances are enabling investigators to probe the nature of consciousness itself. They are also revolutionizing the science of anaesthesiology[5] — allowing investigators to better understand how anaesthetics produce unconsciousness[6], how these mechanisms overlap with natural sleep[7], and how people recover consciousness after anaesthesia[8]. Research into consciousness and anaesthesia overlaps, too, because anaesthetics provide a powerful, reliable means of eliciting reversible states of altered consciousness. Understanding the neural mechanisms of these altered states might lead to fresh approaches to modulate consciousness and control pain without the undesirable side effects of currently available drugs, which include changes in heart rate and blood pressure, cessation of breathing, delirium and nausea.

The complex state of dissociation can be fully described only by humans, who can report their experience. For example, a study in humans was needed to prove that the dissociative and analgesic properties of ketamine are independent[9]. Going forward, studies that use dissociative drugs in people will continue to be of great interest — for instance, to reveal the connection (if any) between the brain rhythm reported by Vesuna *et al.* and the various desirable properties of ketamine. Such studies should also include medicines, such as benzodiazepines and lamotrigine, that attenuate ketamine-induced dissociation. An improved understanding of how ketamine alters brain rhythms and associated behavioural states could eventually lead to therapeutics for people experiencing chronic pain, depression and perhaps dissociative disorders.

These analyses will be highly challenging to perform, because studying deep cortical rhythms requires people in whom intracranial electrodes have been implanted. For ethical reasons, only individuals who require electrodes for therapeutic purposes can participate in such studies. We owe them a debt of gratitude for allowing us to better understand the inner workings of the human brain.

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Drug discovery

Modular synthesis enables molecular ju-jitsu

Daniel J. Blair & Martin D. Burke

An ancient resistance mechanism poses a problem when using streptogramin antibiotics. A modular approach to drug synthesis exploits this same mechanism to generate an antibiotic that avoids the emergence of resistance. **See p.145**

The development of resistance to antibiotics by microorganisms is a problem that has been billions of years in the making, but we don't have as long as that to solve it[1,2]. One way to speed up the search for solutions is to harness human creativity and modern science to rationally design resistance-evasive variants of naturally occurring antibiotic molecules[3,4]. Unfortunately, such molecules often have to be synthesized from scratch using long, highly customized sequences of reactions that are prohibitively slow and impractical at large scales. Li *et al.*[5] report on page 145 how a modular synthesis of the structurally complex antibacterial compound virginiamycin M2 (VM2), based on easily interchangeable molecular building blocks, has provided access to VM2 derivatives that could not previously have been prepared — and has thereby enabled the rational development of a variant that evades an ancient resistance mechanism.

"Replacement of a methyl group with a larger group yielded a compound with potent activity against a series of bacterial strains."

Virginiamycin M2 belongs to the streptogramin family of antibiotics, which is subdivided into groups A and B. The two groups work synergistically to inhibit bacterial protein synthesis by binding to complementary sites in the catalytic centre of the bacterial ribosome (the molecular machinery that coordinates protein synthesis). Group A streptogramins, such as VM2, bind to part of the ribosome called the peptidyl transferase centre (PTC), and promote the binding of group B streptogramins to the adjacent tunnel region, through which nascent proteins exit.

A key mechanism of bacterial resistance to this powerful antibiotic 'one–two punch' probably evolved in parallel with the streptogramins, in the form of acetyltransferase enzymes of the Vat family (VatA enzymes). These enzymes deactivate group A streptogramins by transferring an acetyl group ($-COCH_3$) to an alcohol group ($-OH$) attached to a specific site in the antibiotics, dubbed the C14 position[6]. The addition of an acetyl group produces a molecular bump that clashes with ribosome-bound RNA in the PTC, and thus blocks antibiotic activity (Fig. 1).

There have been several attempts to prepare derivatives of group A streptogramins that could avoid this deactivation mechanism[7]. But, in each case, the derivatives were limited to those that could be prepared from the natural product itself, through a process called semi-synthesis, and were found not to be resistant to VatA-mediated deactivation. Researchers from the same group as Li and colleagues previously developed[8] a highly modular synthesis

of VM2 to enable the unconstrained design of molecular variants. This Lego-like synthetic strategy allows molecular building blocks to be readily swapped, thus providing access to many targeted derivatives.

Such modularity is a powerful enabler of creativity: because many variants can be rapidly assembled and tested, researchers can make almost any target that they want. Moreover, unlike conventional small-molecule synthesis, the price of failure is not high – it doesn't matter if any given compound lacks the desired biological activity, because many others can be prepared, thereby increasing the likelihood of finding active small molecules. Modular construction strategies have previously been implemented on automated systems to make biopolymers such as peptides, oligonucleotides and, increasingly, oligosaccharides. Achieving this modularity in the synthesis of structurally complex small molecules (such as those prepared by Li and colleagues) is a promising step towards the more widespread adoption of modularized small-molecule synthesis[9].

In the present work, unconstrained in their capacity to prepare many different VM2 analogues, Li and co-workers have used modern structural-biology techniques to investigate the molecular basis of VatA-mediated antibiotic resistance. They used cryo-electron microscopy to obtain structures of VM2 and of a series of VM2 derivatives bound to the ribosome of the bacterium *Escherichia coli*. The structures revealed that these compounds bind the PTC, as expected, and that although the C14 alcohol group (the site that is acetylated by VatA) engages in key interactions with nearby components of the ribosome, a methyl group (CH_3) at another position in the compounds (the C4 position) does not.

A previously reported crystal structure[10] of the VatA binding pocket suggested that the C4 methyl group forms close binding interactions with the enzyme. This led Li and co-workers to predict a form of molecular ju-jitsu: flip VatA's resistance strategy back on itself by making a molecular bump at C4 that blocks VM2 binding to VatA and thus mitigates resistance, but which minimally disrupts VM2's binding to the ribosome and therefore maintains antibiotic activity (Fig. 1).

Modifications at the C4 methyl group would be challenging to accomplish by semi-synthesis, but were sitting ducks for Li and colleagues' modular approach. The researchers prepared many derivatives of VM2, including variants of a more potent analogue called flopristin that had previously been evaluated as a drug candidate in clinical trials[11]. Replacement of the C4 methyl group with a larger allyl group yielded a new compound with potent activity against a series of bacterial strains, resistant to VM2 and flopristin, that harboured genes encoding VatA or related enzymes.

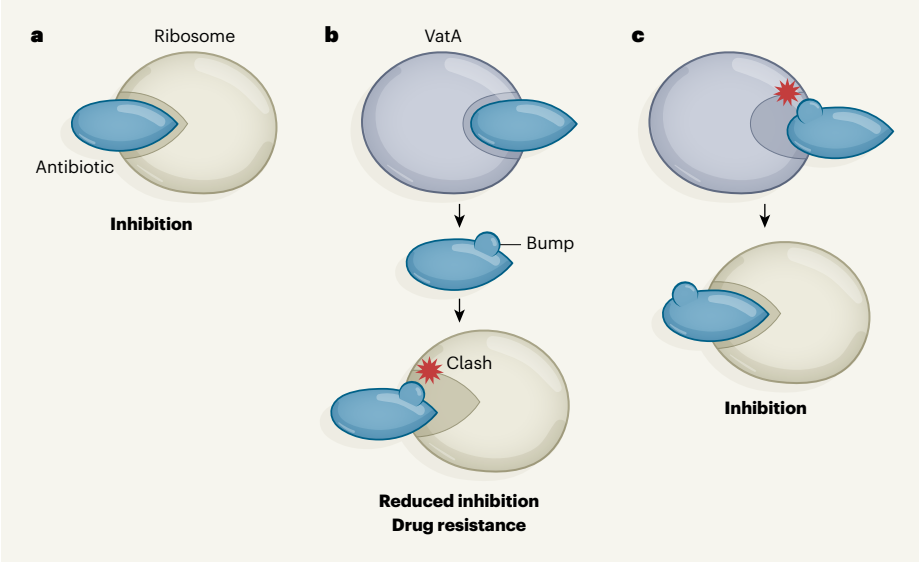

Figure 1 | Overcoming a mechanism of drug resistance. a, Antibiotics known as group A streptogramins bind to a pocket in the bacterial ribosome (the cellular machinery that synthesizes proteins), thus inhibiting ribosomal activity, and so killing bacteria. **b**, The bacterial enzyme VatA adds a molecular 'bump' (an acetyl group) to streptogramins at a particular position. This prevents the modified antibiotics from binding to the bacterial ribosome, thereby leading to drug resistance. **c**, Li *et al.*[5] used a modular strategy for synthesizing streptogramins to find a molecule that mirrors the mechanism used in **b** to evade drug resistance. The addition of a bump (an allyl group) at another part of the antibiotic disrupts binding to VatA, preventing the addition of the resistance-causing acetyl group, but does not disrupt binding to the ribosome.

Further cryo-electron microscopy studies showed that the C4 allyl group does not interfere with the binding of the compound to the ribosome PTC. In fact, the allyl group points towards the binding site for group B streptogramins in the exit tunnel, and seems to make favourable contacts with three nucleotides in the tunnel that are not contacted by VM2. This potentially boosts the overall strength of binding interactions between the allyl-containing compound and the ribosome, and suggests that other compounds that bind more optimally could be made.

Li *et al.* also obtained an X-ray crystal structure of the new compound bound to VatA, which revealed a potential clash between the allyl group and an amino-acid residue (designated Leu 110) in the enzyme. Biochemical assays further showed that, although flopristin and the allyl-containing compound both potently inhibit ribosomal activity, there is a 60% decrease in the rate at which VatA adds an acetyl group to the new compound relative to flopristin. Collectively, these studies provide intriguing evidence that the authors' molecular ju-jitsu strategy was successful.

Li and co-workers went on to test the allyl-containing compound in a mouse model of streptogramin-resistant bacterial infection. Impressively, not only was this compound significantly more effective at inhibiting the growth of VM2-resistant bacteria than was flopristin, but also it required no co-treatment with a group B streptogramin. Many more studies are needed to determine whether the new compound has clinical potential, but these initial results represent an exciting take-off point for further development.

Li and colleagues' study exemplifies how modular chemical synthesis can enable molecular innovation. It also opens the door to the more rational development of group A streptogramins that mitigate VatA-mediated resistance while retaining potent antibiotic activity (see go.nature.com/3huzzej). More broadly, this story will embolden the pursuit of modular, complex small molecules as engines for pushing the frontiers of chemical biology and drug discovery.

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