engineering magnetic interactions between individual units. In general, the main strategy for controlling such swarms relies on the response of the units to remotely controlled global fields, such as magnetic fields[7,8]. Although it is then difficult to deal with each unit individually and locally, collective coupled interactions between the units can be globally controlled, resulting in programmable local interactions, self-assembly and collective behaviour. This method has been used to attain collective two-dimensional assembly, disassembly and manipulation of synthetic microrobotic swarms at the interface between air and water[9].

Li and colleagues' particle-robotics system, and most other collective robotic systems, work mainly in two dimensions. Extending such systems to three dimensions, with more-complex locomotive behaviour of components and their aggregates on surfaces or inside fluids, would increase their possible future applications. However, going to three dimensions would bring many hardware-design challenges for robust locomotion, aggregate stability, reversible and programmable component-attachment methods, miniaturization and control.

In the near future, it will be crucial to demonstrate potential high-impact engineering and medical applications of such collective robotic systems that would be impossible using other techniques. For example, swarms of stochastic bacterium-driven microrobotic swimmers could use the particle-robotics approach to deliver drugs to targeted, hard-to-reach regions inside the human body. Such swarms might, for example, be directed by the chemical gradients, oxygen gradients or changes in pH of cancerous-tissue environments[10]. Indeed, many studies[11,12] have already shown that collective bacterium-driven microrobotic swarms have potential applications in targeted drug delivery, medical diagnostics and environmental sensing. ■

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MEDICAL RESEARCH

Fighting cystic fibrosis with small molecules

In cystic fibrosis, ion-transport abnormalities cause problems in many organs. A small molecule that forms cell-membrane pores allowing ion transport shows therapeutic promise in human cells and a model of the disease. SEE LETTER P.405

DAVID N. SHEPPARD & ANTHONY P. DAVIS

In cystic fibrosis, abnormalities in the ion-channel protein CFTR cause problems in the transport of chloride (Cl^-) and bicarbonate (HCO_3^-) ions in the epithelial cells that line the airways of lungs, resulting in a build-up of mucus in these airways. This hinders the normal process that removes mucus and the inhaled bacteria trapped in it, and the resulting airway blockage leads to persistent infections and inflammation, which destroy lung tissue[1]. On page 405, Muraglia *et al.*[2] demonstrate that a small molecule called amphotericin B, which can form an ion channel in the cellular membrane of airway cells, restores ion transport and antibacterial defences when tested *in vitro* in human cells from people with cystic fibrosis and in an *in vivo* animal model of the disease.

Much progress has been made in developing new treatments for cystic fibrosis, and cocktails of three drugs that might slow disease progression are now in clinical trials[3,4]. A common defect in cystic fibrosis is the failure of CFTR to reach its location on the cell membrane, and two of the drugs help the protein to overcome this, with the third boosting ion transport through the channel. However, approximately 1,800 faulty versions of the CFTR-encoding gene associated with the disease have been identified so far, and this diversity of mutations might mean that drugs targeting CFTR will not work for everyone who has the disease[1].

Interest has therefore grown in trying to find widely applicable treatment options for cystic

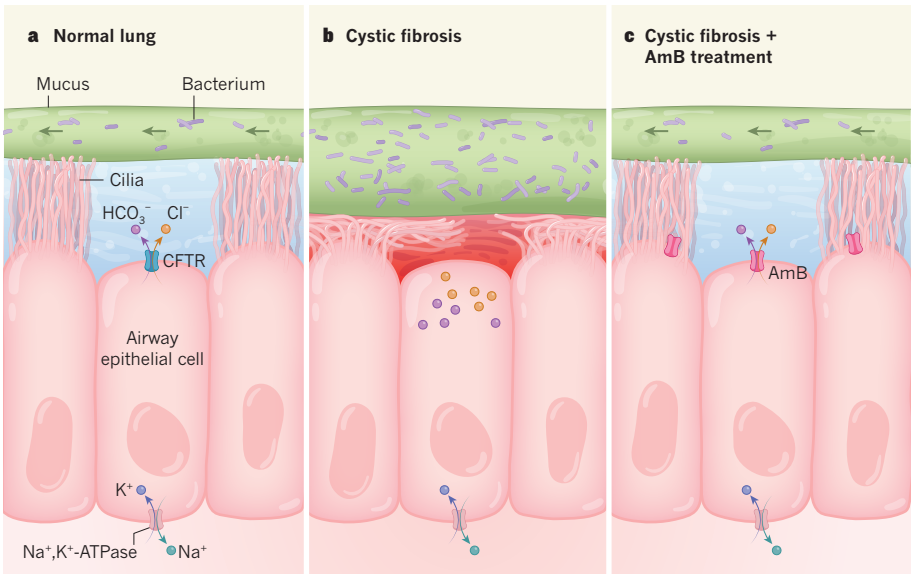

Figure 1 | Amphotericin B tackles lung problems in cystic fibrosis. **a**, Airway epithelial cells in lungs are bathed in a thin layer of airway-surface liquid (blue) on which mucus floats. The beating of cellular protrusions called cilia propel (green arrows) this mucus and trapped bacteria from the airways. Mucus removal, the killing of trapped bacteria and the maintenance of a normal volume of airway-surface fluid at physiological pH require the presence of bicarbonate (HCO_3^-) and chloride (Cl^-) ions in the surface liquid. These ions are secreted into the liquid through the activity of a network of ion-transporting cellular proteins (not all shown), including the Na^+, K^+-ATPase protein, located in the cell's tissue-facing (basolateral) membrane, and the protein CFTR, which is found in the liquid-facing (apical) membrane of certain airway epithelial cells and transports HCO_3^- and Cl^- out of the cell. **b**, In cystic fibrosis, CFTR is absent from some epithelial cells. This causes a build-up of cellular HCO_3^- and Cl^-, and the subsequent formation of airway-surface fluid that is more acidic (red) and of a smaller volume than normal[1,8]. The mucus is thicker than usual, and mucus removal and bacterial killing are abnormal[8]. **c**, Muraglia *et al.*[2] demonstrate in human cells and in a pig model of cystic fibrosis that treatment with the small molecule amphotericin B (AmB), which forms a non-selective ion channel, restores ion transport when CFTR is absent.

fibrosis. For example, efforts are being made to restore ion transport in ways that bypass faulty or missing CFTR proteins, by using other pathways to transport negatively charged ions (anions) out of the cell. Such options include using anion channels that are found naturally in airway epithelial cells[5], or exploiting synthetic molecules that bind anions selectively and function either as artificial anion channels[6] or as transporters that shuttle anions across the lipid membrane[7].

Bicarbonate ions have crucial roles in lung-defence mechanisms, and abnormalities in HCO_3^- secretion from epithelial cells into the airways underlie many symptoms of cystic fibrosis. Analyses[8] of a pig model of the disease indicate that the absence of normal HCO_3^- transport into the airways prevents bacterial killing by antimicrobial factors and affects the pH and viscosity of the thin layer of airway-surface liquid that covers epithelial cells. This liquid surrounds cellular protrusions called cilia (Fig. 1a) that beat to transport away the overlying mucus[8]. Furthermore, HCO_3^- is essential for untangling mucins, the proteins that make up mucus, during mucus formation[9]. In cystic fibrosis, the airway-surface liquid is more acidic than normal, and there is less of it (Fig. 1b), as a result of the defects in anion secretion[1,8].

The Na^+, K^+-ATPase protein is an ion transport pump located on the tissue-facing (basolateral) membrane of airway epithelial cells. It drives cellular anion transport by regulating ion transport through other transport proteins, ultimately enabling the cellular import of Cl^- and HCO_3^- across the basolateral membrane. In people with cystic fibrosis, these anions accumulate in airway cells. The resulting high cellular concentration of Cl^- and HCO_3^- and low concentration in the airway-surface liquid generate a steep concentration gradient for these anions across the liquid-facing (apical) surface of airway cells that might suffice to drive anion exit without the need for an energy source for transport. Muraglia *et al.*[2] therefore reasoned that a small molecule that acts as a HCO_3^- transporter could exploit this concentration gradient to restore HCO_3^- transport and thus also the defence processes that depend on lung HCO_3^-. But which small molecule should be used?

Muraglia and colleagues focused on an antifungal agent called amphotericin B that is made naturally by bacteria. This small molecule might initially seem a strange choice. It forms non-selective ion channels that are permeable to both anions and positive ions (cations), and it can be toxic to human cells[10]. However, three lines of evidence made a persuasive case for this choice.

First, its antifungal activity, which is due to its ability to extract sterol molecules from lipid membranes, is separate from its function as an ion channel, suggesting that the toxicity issue could be managed by judicious control of amphotericin B concentration[10]. Second, amphotericin B restores the transport of potassium ions in yeast cells that lack a potassium transporter, demonstrating that it can provide a functional replacement for natural transport proteins[11]. Third, Muraglia *et al.* demonstrated that amphotericin B transports HCO_3^- across artificial lipid membranes.

When the authors added amphotericin B to the apical membrane of human airway cells — a standard *in vitro* model system for studying cystic fibrosis — HCO_3^- was secreted from the cells, the pH of the airway-surface liquid rose and the volume of airway-surface liquid was restored to normal (Fig. 1c), compared with the effect in cell samples that did not receive amphotericin B. The authors also tested airway epithelial cells obtained from people whose CFTR represented a range of variants of the protein. The addition of amphotericin B to the apical surface of these cells resulted in an increase in pH, a decrease in the viscosity of the airway-surface liquid and an enhancement of bacterial killing compared with untreated cells. Finally, Muraglia *et al.* demonstrated in an *in vivo* pig model of cystic fibrosis that AmBisome, a pharmaceutical formulation of amphotericin B, increased the pH of the airway-surface liquid compared with the pH in untreated animals. This drug is already licensed for use in the clinic.

"The success of this approach will surely encourage a wider exploration of such uses of small molecules."

Muraglia and colleagues' study and other work[12] offer a proof of concept that small molecules can function as surrogates for defective or deficient transport proteins in human disease. Although a small molecule cannot replicate all the functions of a complex protein, the success of this approach will surely encourage a wider exploration of such uses of small molecules.

Why did amphotericin B work so well? The authors report that inhibiting the Na^+, K^+-ATPase in a human airway-cell model of cystic fibrosis prevented the beneficial effects of amphotericin B treatment. Their finding that the molecule's action in the apical membrane seems to require the activity of ion-transport proteins in the basolateral membrane is an example of what is known as transcellular cross-talk between epithelial membranes[13], in which ion entry and exit through the different surfaces of the cell are regulated to prevent cellular damage. Perhaps one reason for amphotericin B's effectiveness is that it can take advantage of the systems that regulate ion flow through the cell.

Muraglia and colleagues' work raises many questions for future research. For example, how much amphotericin B would be needed to fully restore host defences? Could it be used in combination with drugs that rescue faulty CFTR proteins? And would it be safe

to use amphotericin B routinely throughout an individual's life? As new therapeutic approaches are developed for all people with cystic fibrosis, they might also help people who have other lung conditions that have similar disease characteristics. ■

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BIOCATALYSIS

Enzymes trapped and zapped

Many enzymes cooperate with other proteins and small molecules to function. A strategy that mimics the confinement of such cooperative partners in cells might allow these enzymes to be used in applications outside biological systems.

ALISON NARAYAN

Nature precisely controls thousands of chemical reactions in every cell. Many of these form complex chains of reactions, analogous to a falling line of dominoes: when an enzyme triggers the first reaction of a chain, the other reactions are triggered in sequence. Such reaction cascades can be difficult to recreate outside living systems. Even when the same catalysts are used as those that evolved in nature, it is frequently not possible to achieve the naturally occurring reaction rates when cascades are carried out in a test tube. Writing in *Angewandte Chemie*, Megarity *et al.*[1] report a solution to this problem that mimics a naturally occurring strategy for promoting efficiency: the co-localization of cooperating catalysts.

The use of enzymes to catalyse reactions offers an alternative to using conventional catalysts, and can greatly improve the sustainability, safety and cost of chemical processes[2]. The advantages are amplified when multiple chemical reactions can be conducted sequentially in a single reaction vessel, analogous to the way in which reaction cascades occur in a cell. These advantages drive scientists to design improved platforms for enzymatic reactions, despite the difficulty of reconstituting multienzyme cascade processes outside cells[3].

Biological reactions often require electrons to be shuttled to the catalyst, using several protein and small-molecule carriers that are co-localized in cells. Several strategies can facilitate this electron shuttling in cells, including the engineering of high-affinity interactions between partners in the shuttling pathway[4]. Such interactions can be realized at protein–protein interfaces[5], by the binding of small molecules in enzyme active sites[6] and by compartmentalizing reactants within individual organelles[7]. Combinations of these three approaches are used in cells to form nanoscale reactors, which accelerate reactions compared with the analogous processes occurring freely in solution[8].

Megarity *et al.* envisaged mimicking this natural process by trapping enzymes in synthetic nanoreactors on the surface of a plentiful source of electrons, thus facilitating the electron transport required to power biocatalytic reactions. Researchers from the same group as the current authors had previously reported[9] that an enzyme called ferredoxin–$NADP^+$ reductase (FNR) binds tightly to the pores of electrodes made of indium tin oxide (ITO). In natural systems, FNR accepts two electrons from a protein partner called ferredoxin. The interaction between an electrode and FNR mimics the electrostatics of the ferredoxin–FNR protein–protein interaction. More specifically, the negatively charged surface of the electrode mimics the negatively charged patch of ferredoxin that forms an interface with a positively charged surface of FNR (Fig. 1a).

FNR uses the electrons it receives from ferredoxin to chemically reduce a small molecule known as $NADP^+$ — a cofactor that acts as an electron shuttle. The reduced form of the

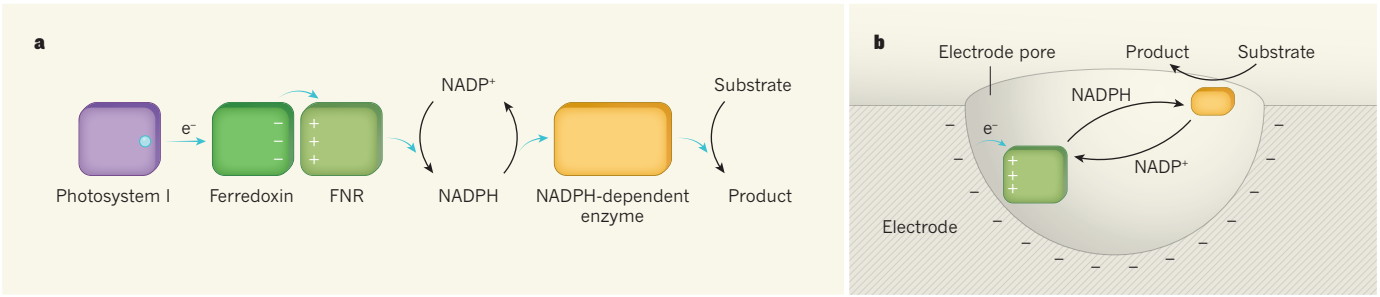

Figure 1 | Enzymatic electron-shuttling processes. a, In photosynthetic systems, a protein complex called photosystem I transfers electrons (e^-) to the ferredoxin protein. Ferredoxin passes electrons to an enzyme called ferredoxin–$NADP^+$ reductase (FNR), to which it binds tightly because a negatively charged region on its surface interacts with a positively charged region on FNR. FNR uses the electrons it receives from ferredoxin to chemically reduce a cofactor, $NADP^+$. The reduced form of the cofactor, NADPH, then transports electrons to an NADPH-dependent enzyme, which uses them to catalyse the transformation of a substrate to a product. **b**, Megarity *et al.*[1] have trapped FNR and NADPH-dependent enzymes in the pores of an electrode, which acts as a plentiful source of electrons. When the system is immersed in a solution of NADPH, electrons flow from the electrode through the cascade of reactions, allowing NADPH-dependent enzymes to be used effectively outside biological systems.