Biotechnology

Mix-and-match tools for protein injection into cells

Charles F. Ericson & Martin Pilhofer

An injection system from bacteria has been re-engineered in an effort to develop a programmable system for protein delivery into cells. Its customizability opens the door to a multitude of biomedical applications. See p.357

Having the ability to deliver particular proteins into specific cell types would offer tremendous potential for research in the life sciences, as well as for the treatment of disease. However, the combined challenges that arise from trying to target cell types of interest and being able to transport proteins of choice across cellular membranes have made the development of such tools a difficult task. On page 357, Kreitz *et al.*[1] report the development of a programmable system for protein delivery that is derived from a molecular injection device found in bacteria. Building on a growing body of knowledge regarding the mechanisms that mediate bacterial interactions with cells, the authors show that this approach can be tuned to target specific cells and to deliver customized protein cargoes (payloads). These re-engineered injection complexes represent an exciting biotechnological toolbox that could have applications in various biological systems.

The protein complex that Kreitz and colleagues chose to modify comes from a family of bacterial protein apparatuses that can be thought of as a type of spring-loaded molecular syringe. These naturally occurring injection devices contain a hollow needle that carries the cargo and punctures the target cell. Using a contractile mechanism, the needle is physically forced to penetrate the targeted cell membrane and project into the cell[2]. These complexes are used by bacteria to inject molecules (termed effector molecules) into neighbouring cells as a way to mediate interactions with cells in their environment.

Studies have highlighted the abundance of evolutionarily conserved gene clusters that encode these systems in diverse species of bacteria[3–7]. From the structural and functional characterization of a small number of these systems, it emerges that bacteria have evolved differing strategies to use them. The major modes of action are the type VI secretion systems that remain bound to the bacterial cell[8], and extracellular contractile injection systems (Fig. 1) that are released from bacteria to act at a distance[6,9–11].

Kreitz *et al.* recognized the potential of re-engineering extracellular contractile injection systems because these are known to have structures (tail fibres) similar to those found in viruses called phages that target bacteria. These structures mediate binding to cell-surface receptors[12]. The authors focused on a particular system known as *Photorhabdus* virulence cassettes (PVCs), which naturally target and kill insect cells[10]. These structures are found in *Photorhabdus* bacteria, which live in association with nematode worms. PVCs proved to be an enticing choice for engineering because they can target mouse cells[13] and previous studies had already laid the groundwork for expressing and purifying PVCs[7].

Harnessing these developments, along with help from the artificial-intelligence program AlphaFold in predicting protein structures, the authors identified a region of the PVC tail fibre that conferred specificity towards insect cells — the system's native target. The predicted globular portion at the end of the tail fibre proved to be responsible for the targeted binding. The authors then replaced this portion of the tail fibre to retarget PVCs towards specific mouse and human cells. This was accomplished by genetically fusing the sequence for a truncated tail fibre with sequences for protein domains that can specifically bind the PVC particles to distinct receptors on the surfaces of cells.

To complement their retargeting efforts, the authors set out to load the PVCs with various protein cargoes. A previous study identified a protein-loading sequence in a segment of the normal PVC cargo protein[14]. Interestingly, this previous study demonstrated that a variety of protein types could be loaded into the particle, regardless of the cargo's size or charge.

Kreitz and colleagues used fusions of this packaging domain as a way to load reporter proteins into retargeted PVCs and tested them using laboratory-grown cell cultures. Specific protein delivery could then successfully be monitored by approaches such as cell-killing tests and direct visualization by

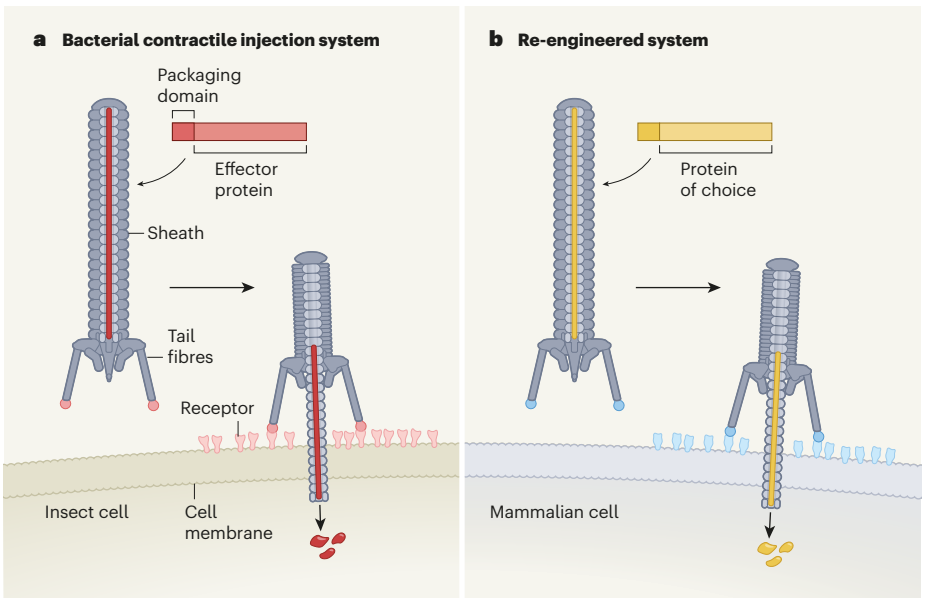

Figure 1 | Re-engineering of a contractile injection system from bacteria. a, *Photorhabdus* bacteria secrete an extracellular structure that enables the injection of various proteins (termed effector proteins) into targeted insect cells. A packaging domain targets proteins to be loaded in the structure's hollow tube. Tail fibres confer specificity towards the targeted cell. Once the fibres come into contact with the target cell, they bind to receptors on the cell surface and the sheath contracts. This action physically pushes the tube through the target membrane, delivering proteins directly into the cell. **b**, Kreitz *et al.*[1] re-engineered this system to develop a protein-delivery tool. They modified portions of the tail fibres to enable targeting of desired cell types. Harnessing a packaging domain enabled the authors to load their proteins of choice. These re-engineered contractile injection systems were able to target and deliver chosen proteins into specific mammalian cell types that are strikingly different from the cells normally targeted by these bacteria.

light microscopy. The authors also succeeded in loading the large Cas9 protein component of the gene-editing system CRISPR, and when these PVCs were supplied to cells with a guide RNA, specific gene editing (base editing) was detected.

Following on from their work on protein introduction to cultured cells *in vitro*, Kreitz and colleagues explored whether the re-engineered PVCs could function in a live animal. Purified PVC particles were injected directly into the hippocampus region of mouse brains. The authors observed the fluorescent signal of the cargo protein only around the injection area. Importantly for future biomedical applications, these PVC injections did not trigger local immune-cell activation and PVCs could no longer be detected seven days after the brain injection.

It was previously suggested that various bacterial secretion systems with the ability to inject molecules in a way that is dependent on cell–cell contact could serve as a tool for delivering cargo to target cells. By comparison, the use of extracellular contractile injection systems has two key advantages. First, their mechanism of conferring specificity is now well understood, which is not the case for bacterially anchored secretion systems. Second, extracellular contractile injection systems are defined assemblies that can be purified and applied to target organisms without the need for a treatment that requires live bacteria or minicells (small engineered artificial bacterial cells that lack chromosomes).

An interesting future direction would be to expand the target range of PVCs to include bacterial cells. Other studies have suggested that related extracellular contractile injection systems called pyocins could be used as antibacterial agents[15,16]. Such efforts would widen the scope of future applications by providing a programmable platform that might overcome a potential issue in existing antibacterial phage therapy — namely, unwanted phage replication[17].

Finally, the approaches developed by Kreitz and colleagues might be useful for studying and engineering other contractile injection systems. Despite their high diversity and multitude of functional roles, the systems characterized so far seem to rely on an evolutionarily conserved core set of components that have relatively minor structural differences that allow for notable mechanistic adaptations. This modular design might be harnessed in the future to try to develop, for example, engineered multi-barrelled complexes that form arrays of many individual contractile injection systems[5,6]. These might enable more cargo to be delivered per target cell than with a single injection system.

The platform of PVCs, offering the reported customizable features of cargo and specificity, might be complemented by other platforms and modules in efforts towards generating a powerful mix-and-match toolbox for research and medicine. Will these systems become commonplace in our labs and lives in the future?

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Materials science

Excited states identified from molecular movies

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A molecular process called singlet fission might boost solar-cell efficiency, but the mechanism must first be determined. A technique that probes molecules undergoing this process finally reveals the excited states involved. **See p.275**

Over the past two decades, a strategy has emerged for turning waste heat produced by widely used solar cells into useful energy, based on a quantum-mechanical phenomenon called singlet fission[1]. On page 275, Neef *et al.*[2] shed light on the steps involved in singlet fission in a molecular semiconductor called pentacene. The findings settle a long-standing debate about the sequence of events and bring us closer to using singlet fission to improve solar-cell efficiencies.

Sunlight is the most abundant source of sustainable and clean energy. However, to produce electricity at the scale needed by society, affordable solar cells are required that convert sunlight to electricity efficiently. Unfortunately, achieving highly efficient, low-cost solar cells is a major challenge, despite decades of effort. This is due to the apparent trade-off between the efficiency of solar cells and their cost and complexity.

For example, conventional 'single junction' silicon solar cells are cheap and simple, but have a fundamental efficiency cap of about 30% (ref. 3). This limit arises because these solar cells are most efficient at converting photons (single particles of light) of a particular energy to electricity, whereas sunlight has a broad energy spectrum. The lower-energy photons in sunlight are not absorbed by silicon, whereas higher-energy ones generate unwanted heat.

So how could singlet fission help? Singlet fission occurs in molecular semiconductors, and describes the splitting of one 'bright' light-induced excitation (a singlet) into two 'dark' excitations of lower energy, called triplets. This process produces two excited electrons from just one photon, and has the potential to greatly increase the output of electric current and the efficiencies of conventional solar cells. The phenomenon of singlet fission was first described[4] in 1965, but it was not until 2004 that the link to solar cells was made[5]. However, its promise in this regard has yet to be realized, with only a few reports showing that the efficiency of silicon solar cells can be enhanced by this phenomenon (see ref. 6, for example).

The main challenge has been developing molecules capable of singlet fission that are suitable for use in silicon solar cells, with a key bottleneck being the limited understanding of the underlying process. Despite decades of research, and widespread agreement that fission does indeed occur and can be amazingly fast (on subpicosecond timescales; 1 ps is 10^{-12} seconds), the fundamental mechanism is still debated. Among the possible phenomena to be implicated so far are quantum-coherent