TechNews

SYNTHETIC BIOLOGY: ENGINEERING ORDER IN ORGANISMS ACROSS SCALES AND SPECIES

BILL JIA AND ARIN WONGPROMMOON EXPLORE ENGINEERING APPROACHES TO CONTROLLING BIOLOGICAL PROCESSES

Synthetic biology has enormous potential to solve problems in health, agriculture, and energy. It can be thought of as the rational manipulation of the information flows carried in DNA, RNA and proteins that dictate form and function to cells. Since the invention of the first synthetic genetic circuits in the year 2000 – the repressilator [1] and the genetic toggle switch [2] – researchers have engineered organisms that can act as sensors, factories and computers.

Many questions must be answered before the products of synthetic biology can be used universally. Can engineered organisms complete tasks with multiple steps or decision points? Can we rationalize and predict their behavior? Are these designs reproducible, and can they be modular? Are they safe enough to carry out their intended function only? Solutions to these problems would be major steps towards the goal of imposing human-defined order onto biological systems.

Impressive progress has been made towards these fundamental challenges. As a result, more complex circuits have been built for a variety of purposes. Furthermore, the biological design space has expanded as researchers have constructed systems in different species, and in populations and communities rather than at the single-cell level. We are now seeing increasingly refined examples of the promise of engineered organisms for the real world.

Orthogonal signaling for spatial patterning

Spatial organization is prominent in all cellular biology, from bacterial biofilms to mammalian embryos. Patterning allows division of labor for more complex behavior, and protection from harsh environments. Jim Haseloff's team

in the Department of Plant Sciences at the University of Cambridge (UK) recently demonstrated a synthetic genetic circuit that allows a population of bacteria to display three distinct phenotypes in space [3].

The system relies on homoserine lactones (HSLs), small molecules that induce gene expression in many types of bacteria. HSLs freely diffuse into and out of membranes, meaning that their environmental concentration indicates the relative number of nearby cells. This results in quorum sensing behavior in natural populations. HSLs have been popular amongst synthetic biologists for engineering cell-cell communication. However, it can be difficult to carry more than one channel of information using multiple HSLs because their high degree of evolutionary conservation creates cross-talk between variants.

The team at the University of Cambridge engineered a set of synthetic promoters that greatly reduces signaling interference between two different HSLs derived from *Vibrio fischeri* and *Pseudomonas aeruginosa* (3OC6HSL and 3OC12HSL, respectively). Here they used the newly orthogonal signals as inputs to an "AND gate" circuit housed in *Escherichia coli*, to control the expression of three different fluorescent proteins: yellow (YFP), cyan (CFP) and red (RFP).

CFP and YFP expression were directly controlled by 3OC6HSL and 3OC12HSL, respectively. In contrast, RFP was only expressed in the presence of both signals because it was placed under the control of a T7 promoter. Transcription from this promoter requires T7-RNAP, a non-native RNA polymerase. In the engineered *E. coli* strain, each HSL-associated promoter controls the production of one nonfunctional T7-RNAP fragment. When they are both made, they fit together like puzzle pieces and can transcribe from the promoter, displaying the logical AND gate behavior (Figure 1).

When the bacteria were subjected to opposing gradients of the two HSLs, RFP was only expressed in a ‘sweet spot’ where they were both present at moderate levels, resulting in a tricolor pattern (Figure 2). The authors then gave two populations of bacteria the additional ability to produce either 3OC6HSL or 3OC12HSL independently. Amazingly, the bacteria coordinated the same pattern without any external HSL gradients when cultured next to each other.

The authors’ system could be easily extended to form increasingly complex hierarchical patterns. If another signaling molecule were to be placed under the control of the T7 promoter, it could act as an input to two additional AND gates – one each with 3OC6HSL and 3OC12HSL, creating two additional distinct spatial regions. As long as suitable orthogonal signals can be found, this algorithm could be repeated to even further segment the initial population of bacteria.

Lead author Christian Boehm explained that in nature, “self-organization in multicellular organisms is shaped by hierarchical induction of gene expression domains. Our work demonstrates how such processes can be emulated using a rationally designed synthetic genetic circuit, opening a door to a higher level of control over multicellular behavior” [4].

Hierarchical patterning could also be a promising route to control multi-step chemical reactions required for production of pharmaceuticals or biofuels. In many cases, dividing enzyme expression spatially could prevent issues with toxicity or genetic incompatibility in different steps, increasing production efficiency and quality of the end product.

Figure 1. AND gate gene circuit for hierarchical patterning. Expression of both 3OC6HSL and 3OC12HSL (bottom) is needed for RFP expression (top). Used with permission from [3]. RFP: Red fluorescent protein.

Figure 2. Gene circuit fluorescent reporter expression in the presence of HSLs. (A) CFP and YFP respond to each individual HSL, while RFP is expressed in the presence of both. **(B)** Under opposing gradients of 3OC6HSL and 3OC12HSL, there are distinct regions where each color is most prominent. Used with permission from [3]. CYP: Cyan fluorescent protein; HSL: Homoserine lactone; RFP: Red fluorescent protein; YFP: Yellow fluorescent protein

Synthetic communities

Recently, microbial ecosystems such as the gut microbiome and plant rhizosphere have received much scientific attention. Many properties of biological systems emerge not from individual organisms, but from the sum of interactions between members of a community. For example, microbial communities can produce a wider range of metabolic products than individual species, and are more resistant to environmental perturbations. These advantages and opportunities represent a frontier for synthetic biology, but manipulation of the complex interactions between strains and species is a great challenge.

To shed light on the dynamics of microbial communities, scientists at the University of Illinois at Urbana-Champaign’s Department of Bioengineering (IL, USA), led by Ting Lu and James J Collins (Massachusetts Institute of Technology, MA, USA), have developed synthetic genetic circuits that re-capitulate six two-strain relationships commonly present in ecosystems (Figure 3) [5]. The interactions were realized using nisin and lactococcin A, two antimicrobials from the bacterium *Lactococcus lactis*. Nisin also behaves as a quorum-sensing molecule, allowing it to effect positive interactions.

The interaction of *L. lactis* with each antimicrobial is defined by a gene cluster, with independent modules responsible for production, translocation and immunity. The authors realized the individual interactions by including only specific modules in each designer bacterium. For example, commensalism was achieved by having one strain express tetracycline resistance (*tetR*) and nisin constitutively. The other strain was equipped

with only the signaling and immunity modules of the nisin gene cluster, and a nisin-inducible *tetR* gene. In other words, the second strain’s tetracycline resistance was dependent on nisin signaling from the first. As a consequence, the first strain was able to survive on its own when exposed to tetracycline, but the second was dependent on the first in the same environment.

The authors then developed mathematical models predicting the expression of each relevant gene and the ecological dynamics. The models consider the populations of each strain in a pair, nisin and lactococcin A levels, and the nutrient concentration. The team then tried to predict the dynamics of three- and four-strain ecosystems by combining their models for the pairwise interactions. The models generally agreed with experimental measurements (Figure 4), suggesting that community dynamics can be predicted by studying the properties of simpler social interactions. In the publication, the authors write:

“Social-interaction engineering also enables the establishment of synthetic

Figure 3. Two-strain synthetic ecological interactions. The authors engineered bacterial strains that in pairs emulated social interactions. Pointed arrowheads indicate a positive effect on population, while flat arrowheads indicate a negative effect. Used with permission from [5].

ecosystems with predictable temporal and spatial dynamics, which can serve as a controllable tool to systematically perturb microbiomes and further alter their structure and dynamics in a desired manner."

Finally, the researchers studied the spatial dynamics of the symmetrical interactions (neutralism, cooperation and competition). They extended their models to consider diffusion through space, and then validated them experimentally. As predicted by the model, the strains within individual colonies seeded on an agar plate were randomly distributed in neutralism and colocalized in cooperation, while only one strain survived in competition.

The study "provides fundamental insights into the structure, dynamics and ecology of interacting microbial species by characterizing synthetic consortia that serve as well-defined abstractions of native communities," the authors said.

The results additionally suggest that synthetic communities for real tasks could be constructed in a systematic way. In the production of chemical products, division of labor could be

controlled through cooperation or commensalism, and one of the negative interactions could regulate the amount of output.

Engineering order for well-being

Synthetic biology also finds its place in promising *in vivo* therapeutic strategies. Martin Fussenegger at ETH Zürich and Nina Khanna at the University of Basel (both Switzerland), along with their colleagues, used synthetic biology to engineer cells that short-cut the flow of information in the mammalian immune system to thwart methicillin-resistant *Staphylococcus aureus* (MRSA) infections [6].

Fussenegger says that their study, as published in *Cell* earlier this year, "provides a first-of-its-kind cell-based therapy for diagnosis, prevention, and cure of one of the planet's most devastating pathogens."

MRSA is associated with implant-associated infections, accounting for half of the two million cases of hospital-borne infections in the USA each year, and 19,000 deaths – greater

Figure 4. Three-strain ecological interactions predicted by two-strain dynamical models. Experimental results for each strain's population density (OD_{600}) are shown in circles, while the model predictions are marked as lines. The models could predict different equilibria between strains as well as extinction events in eight different configurations.

Used with permission from [5].

Figure 5. Human cells engineered to detect and destroy MRSA. Infect_{pro} cells contain a signal transduction system and a genetic circuit that enables them to detect molecules derived from MRSA through Toll-like receptors and produce lysostaphin to kill them. These cells are encased within microcapsules in a tissue cage. In mice models, they are able to diagnose, prevent and treat MRSA infections.

Used with permission from [6].

than influenza, hepatitis and AIDS combined. The gold-standard in treatment involves the administration of vancomycin, a second-line antibiotic that does not always work and can have serious side-effects.

However, cell-based therapy can offer an alternative to antibiotics for such life-threatening bacterial infections, Khanna says. The group developed synthetic human cells that contain a synthetic gene circuit, Infect_{sen} to sense and diagnose MRSA infections and Infect_{pro} to detect and kill the MRSA bacteria. The cells carry out these processes while being shielded from host immune reactions to foreign bodies in microcapsules (Figure 5).

When a pathogen like MRSA enters the body, the innate immune response serves as an initial line of defense against the pathogen. First, hallmark molecules derived from the pathogen are detected by toll-like receptors, which are on the surface of epithelial cells or innate phagocytic cells. This reception starts a signaling cascade in the cell that leads to the secretion of pro-inflammatory cytokines and chemokines. Both of these classes of signaling molecules recruit immune cells called phagocytes, which then engulf the pathogen by phagocytosis, thereby preventing the pathogen from causing harm.

Fussenegger's group modified this information flow by creating a shortcut: they rewired the Toll-like receptors directly to

the expression of a highly effective antimicrobial enzyme, condensing the entire process into a single cell. The enzyme lysostaphin was chosen as it was reported to be more efficient at killing bacteria than broad-spectrum antimicrobials, including vancomycin. This engineering feat also combats MRSA's strategies to evade the immune response, which includes blocking chemotaxis and resisting phagocytosis.

"Such a closed-loop gene circuit was shown to equip mammalian cells with fast and effective anti-infective properties that are otherwise unrealizable" said Ying Liu, a doctoral student in Fussenegger's research group.

When they put the system they engineered to test *in vivo*, the results were stunning.

"The results show that our designer cells can not only diagnose infection, but also prevent the infection from occurring at the first place. Furthermore, we showed that our cells were able to cure 100% of bacterial infections and biofilm formation in mice," commented Liu.

Infect_{pro} was shown to have a 91% prevention rate and the levels of lysostaphin returned to basal levels after elimination of infection. The treatment efficiency far outstripped recombinant lysostaphin and the conventional antibiotic vancomycin, which had cure rates of only 38%.

However, the use of protein-based approaches as employed in the Infect_{pro} system has unresolved issues, such as the stability of the protein. Injecting lysostaphin alone was not sufficient, and the group suspected that it was due to the poor pharmacokinetics of the enzyme. Infect_{pro} cells delivered in microcapsules therefore may serve as a method to overcome this issue by enabling infection-targeted drug delivery and access to difficult-to-reach regions, such as bone prostheses. Although there are more challenges to overcome before Infect_{pro} can be a viable solution to the antibiotic resistance epidemic, Khanna is optimistic.

"We hope within the next 10 years to bring this study into human clinical trials and to provide a new way to tackle infections and antibiotic resistance," she concluded.

Recent developments in synthetic biology show the great potential of this field. Haseloff and his colleagues engineered bacteria to use diffusible signal molecules to create logic gates. As they exhibit orthogonality, their system can lead to synthetic hierarchical patterns, which would aid the construction of multi-cellular systems used in biotechnology and medicine.

Synthetic microbial communities built by Collins and Lu provided insight into the dynamics of natural ecosystems. This may help us take advantage of interspecific interactions to optimize production of chemicals, or to repurpose natural communities.

On the therapeutic side, Fussenegger and Khanna designed cells that detect and destroy virulent bacteria far better than conventional methods of drug administration, and may become a product used in hospitals in the near future. Judging from these three studies, synthetic biology is becoming an instrumental tool in solving problems across biological disciplines.

References

1. Elowitz MB, Leibler S. A synthetic oscillatory network of transcriptional regulators. *Nature* 403, 335–338 (2000).
2. Gardner TS, Cantor CR, Collins JJ. Construction of a genetic toggle switch in *Escherichia coli*. *Nature* 403, 339–342 (2000).
3. Boehm CR, Grant PK, Haseloff J. Programmed hierarchical patterning of bacterial populations. *Nat. Commun.* 9, 776 (2018).
4. New paper out now! Programmed hierarchical patterning of bacterial populations. *Synthetic Biology in Cambridge* (2018). www.synbio.cam.ac.uk/news/programmedpatterning
5. Kong W, Meldgin DR, Collins JJ, Lu T. Designing microbial consortia with defined social interactions. *Nat. Chem. Biol.* 14, 821–829 (2018).
6. Liu Y, Bai P, Woischnig AK *et al.* Immunomimetic designer cells protect mice from MRSA infection. *Cell* 174, 259–270 (2018).

Written by Bill Jia & Arin Wongprommoon

Cambridge University Synthetic Biology Society, University of Cambridge, CB2 3EA, UK

BioTechniques 65: 113–119 (September 2018)
10.2144/btn-2018-0121

Nanoject III The Smallest Big Deal in Microinjection

Completely programmable nanoliter injection control expands your capabilities

- User-friendly touchscreen setup for single injections as well as multiple injection cycle recipes
- Advanced hydraulics deliver precise, consistent injection volumes down to 0.6 nanoliters
- Improved chuck design provides simple, secure micropipet attachment without the need for O-rings

Go to **injection.expert** for more details about Nanoject III expanded microinjection capabilities.

**Nanoject III...
a Big Deal
in Microinjection**

Developers of the original Pipet-Aid®

500 Parkway, Box 700 • Broomall, PA 19008 • 800.523.7480