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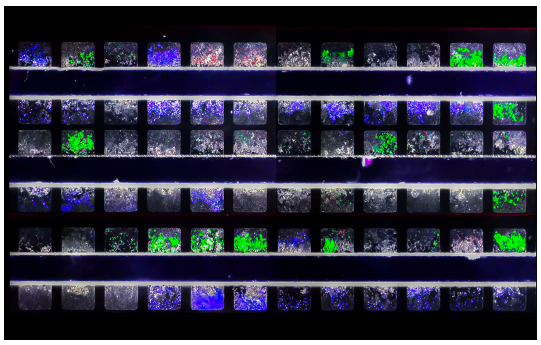
Synthetic Biologists Extend Functional Life of Cancer-Fighting Circuitry in Microbes

Bioengineers and biologists at the University of California San Diego have developed a method to significantly extend the life of gene circuits used to instruct microbes to do things such as produce and deliver drugs, break down chemicals and serve as environmental sensors.



A still image pulled from UC San Diego video from three-strain co-culture experiments of E. coli. The video the image is from demonstrates autonomous cycling of the researchers' synchronized lysis circuit (SLC) that causes microbe population lysis once a threshold population density is reached. Image taken at 10X magnification. The three strains are mixed prior to loading into the microfluidic device. Strains compete until a single strain remains in each trap.

Most of the circuits that synthetic biologists insert into microbes break or vanish entirely from the microbes after a certain period of time—typically days to weeks—because of various mutations. But in the September 6, 2019 issue of the journal *Science*, the UC San Diego researchers demonstrated that they can keep genetic circuits going for much longer.

The key to this approach is the researchers' ability to completely replace one genetic-circuit-carrying sub-population with another, in order to reset the mutation clock, while keeping the circuit running.

"We've shown that we can stabilize genetic circuits without getting into the business of fighting evolution," said UC San Diego bioengineering and biology professor Jeff Hasty, the corresponding author on the study. "Once we stopped fighting evolution at the level of individual cells, we showed we could keep a metabolically-expensive genetic circuit going as long as we want."

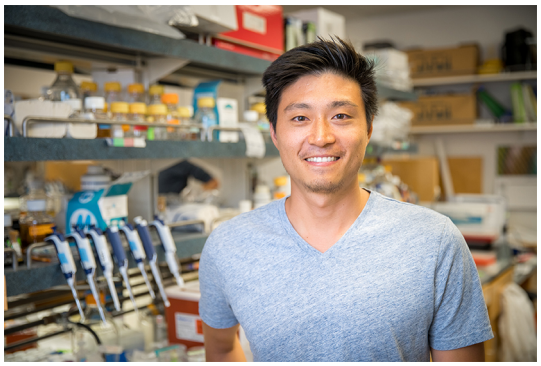
The circuit the UC San Diego researchers used in the *Science* study is one that this team, and others, are actively using to develop new kinds of cancer therapies.

“As synthetic biologists our goal is to develop gene circuits that will enable us to harness microorganisms for a wide range of applications. However, the reality today is that the gene circuits we insert into microbes are prone to fail due to evolution. Whether it be days, weeks, or months, even with the best circuit-stabilization approaches, it’s just a matter of time. And once you lose functionality in your genetic circuit, there is nothing to do but start over,” said Michael Liao, a UC San Diego bioengineering PhD student and the first author on the *Science* paper. “Our work shows there is another path forward, not just in theory, but in practice. We’ve shown that it’s possible to keep circuit-busting mutations at bay. We found a way to keep hitting reset on the mutation clock.”

If the team’s method can be optimized for living systems, the implications could be significant for many fields, including cancer therapy, bioremediation, and bioproduction of useful proteins and chemical components.

Rock Paper Scissors

To actually build a “reset button” for the mutation clock, the researchers focused on dynamics between strains of microbes, rather than trying to hold selective pressures at bay at the level of individual cells. The researchers demonstrated their community-level engineering system using three sub-populations of *E. coli* with a “rock-paper-scissors” power dynamic. This means that the “rock” strain can kill the “scissors” strain but will be killed by the “paper” strain.



Photographs of first author Michael Liao, a bioengineering PhD candidate at the University of California San Diego Jacobs School of Engineering.

Most published work tends to focus on stabilization strategies that act at the level of single cells. While some of these approaches may be sufficient in a given therapeutic context, evolution dictates that single cell approaches will naturally tend to stop working at some point. However, since the rock-paper-scissors (RPS) stabilization acts at a community level, it can also be coupled with any of the systems that act on a single cell level to drastically extend their lifespan.

Making Cancer Drugs and Delivering them to Tumors

In 2016 in *Nature*, UC San Diego researchers led by Hasty, along with colleagues at MIT, described a “synchronized lysis circuit” that could be used to deliver cancer-killing drugs that are produced by bacteria that accumulate in and around tumors. This led the UC San Diego

group to focus on the synchronized lysis platform for the experiments published in *Science*.

These coordinated explosions only occur once a predetermined density of cells has been reached, thanks to “quorum sensing” functionality also baked into the genetic circuitry. After the explosion, the approximately 10% of the bacterial population that did not explode starts growing again. When the population density once again reaches the predetermined density (more “quorum sensing”), another drug-delivering explosion is triggered and the process encoded by the researchers’ synchronized lysis circuit restarts.

The challenge, however, is that this cancer-killing genetic circuit – and other genetic circuits created by synthetic biologists – eventually stop working in the bacteria. The culprit. Mutations driven by the process of evolution.

“The fact that some bugs naturally grow in tumors and we can engineer them to produce and deliver therapies in the body is a game-changer for synthetic biology,” said Hasty. “But we have to find ways to keep the genetic circuits running. There is still work to do, but we’re showing that we can swap populations and keep the circuit running. This is a big step forward for synthetic biology.”

Biomedical Research Advances

One of the research teams working to further advance and implement the synchronized lysis circuit is run by Tal Danino, now a professor at Columbia University, who also published seminal work on the development of quorum sensing for synthetic biology as part of his Ph.D. at UC San Diego.

“Tal recently showed that synchronized lysis technology can be used to deliver an immunotherapy to tumors in mice. To my knowledge, they are the first to show that bacterial drug production and delivery within a treated tumor can modify the immune system to attack untreated tumors. The results are fascinating. They also highlight how important it is for us to figure out how to keep the lysis circuit running as long as possible,” said Hasty.

The current approach is not limited to a three-strain system. Individual sup-populations of microbes, for example, could each be programmed to produce different drugs, offering the potential of precise combination drug therapies to treat cancer, for example.

The researchers studied the dynamics of the populations using microfluidic devices that allow for controlled interactions between the different sub-populations. They also demonstrated the system is robust when tested in larger wells.

One next step will be to combine the approach with standard stabilizing approaches and demonstrate the system works in live animal models.

“We are converging on an extremely stable drug delivery platform with wide applicability for bacterial therapies,” said Hasty.

Hasty, Din, and Danino are co-founders of GenCirq, a company which seeks to transfer this and related work to the clinic.

“Stabilized genetic constructs through engineered synthetic communities,” in the 6 September 2019 issue of the journal *Science* by Michael J Liao1;2, M. Omar Din2, Lev Tsimring 2;3, Jeff Hasty 1;2;3;4

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Competing Interests
A patent application (U.S. provisional patent application 62/682,755) has been filed on the multistrain population control system. J.H. and M.O.D. have a financial interest in GenCirq, a company which seeks to commercialize this and related work.

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