Gene-OFF switches tool up synthetic biology

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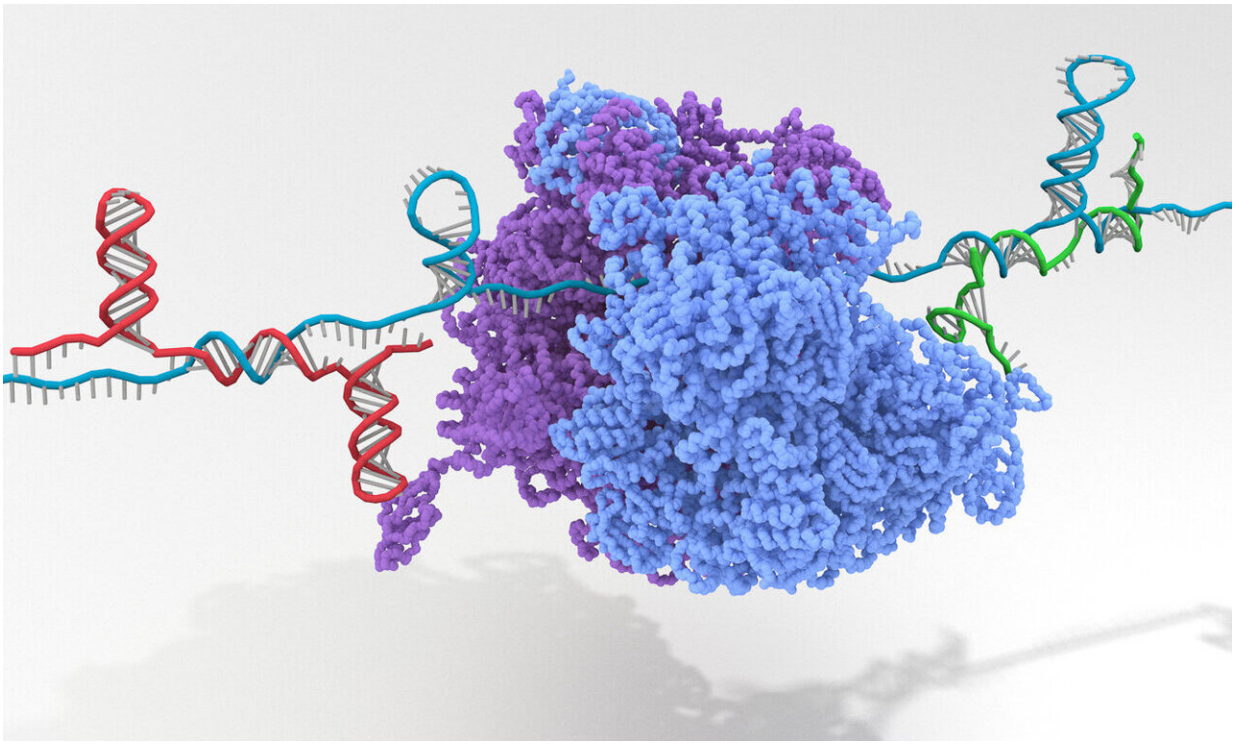

This illustration shows how a complex synthetic RNA containing multiple programmable Riborepressors (long teal blue strand) is bound by incoming trigger RNAs (shorter red and green RNA strands) that change the Riborepressors' secondary structures to block the protein-synthesizing ribosome from accessing its binding sites (purple and light blue structure in the center). Credit: Alexander Green / Arizona State University

In the quest for tomorrow's diagnostics, therapeutics, and bioproduced drugs and fine chemicals, synthetic biologists are assembling artificial

networks of genes and modular regulatory elements, similar to the electronic circuits in computer chips. Introduced into cells, these networks can sense biological signals such as viruses and inflammation markers, or chemical substances, and respond by producing a reporter signal, therapeutic protein, or enzyme that converts one substance into another.

Taking a step forward, a team from Harvard's Wyss Institute for Biologically Inspired Engineering and Arizona State University (ASU), Tempe, recently designed "Ribocomputing devices" that can sense multiple biological RNA signals simultaneously and act as "molecular logic boards." Only if a certain combination of input signals is present, does the device produce a desired output protein. Another desirable regulatory element for synthetic biology would be a device that can do exactly the opposite—effectively shut down the expression of protein in response to a stimulus when it is no longer wanted.

Now, the team together with researchers from Northwestern University, Evanston, Illinois and the Technical University of Munich, has developed two types of programmable repressor elements that can switch off the production of an output protein in synthetic biology circuits by up to 300-fold in response to almost any triggering nucleotide sequence. The researchers created a library of more than 100 repressors to choose from, with up to 15 that can work in parallel in a single circuit. In addition, they combined up to four repressor elements in universal NAND (NOT-AND) and NOR (NOT-OR) gates in complex molecular logic boards computing the presence of multiple incoming nucleotide signals to silence an outgoing fluorescent reporter signal. The study is published in *Nature Chemical Biology*.

"Our Repressor Switch devices add a new capability to the synthetic biology toolbox for researchers designing synthetic biological circuits," said co-corresponding author and Wyss Institute Core Faculty member

Peng Yin, Ph.D. "They have the potential to usher in the possibility of more sophisticated and accurate applications in different areas of next-generation diagnostics, environmental reporting, as well as biomanufacturing." Yin also is Professor of Systems Biology at Harvard Medical School (HMS) and a lead of the Wyss Institute's Molecular Robotics Initiative.

The team previously developed "Toehold Switches", de novo-designed RNA strands that detect trigger RNAs with virtually arbitrary sequences to activate translation of a linked protein-coding RNA sequence into a protein. Complementary regions in the Toehold Switches form a hairpin-like structure that conceals short sequences allowing the ribosome, the molecular machine that translates RNA into protein sequence, to bind to the device and start its job. Incoming trigger RNAs bind to a small "toehold" sequence in the switch, which opens the hairpin up to now allow the ribosome access.

"In our Repressor Switch RNAs, we essentially inverted the function of Toehold Switches using two different strategies. A trigger RNA now induces a structural change in the switch that hides ribosome binding and translation start sites of an encoded protein and thus puts an abrupt stop to protein translation," said Alexander Green, Ph.D., a co-corresponding author of the study together with Yin. "By designing, refining, and studying large libraries of both Repressor Switch elements, we demonstrated that "Toehold Repressor Switches" achieve high dynamic range, allowing us to strongly modulate the production of green fluorescent protein in E. coli bacteria from a very high to a very low level—up to 300-fold." Green, a former Postdoctoral Fellow with Yin, joined ASU's Biodesign Institute and School of Molecular Sciences as Assistant Professor in 2015.

"With slightly lower dynamic ranges, "Three-Way Junction (3WJ) Repressor Switches" could be more effectively combined into complex

modular circuits to sense multiple trigger RNAs without interference from each other," said co-first author Jongmin Kim, Ph.D. who worked as a postdoc in Yin's group and now is Assistant Professor at Pohang University of Science and Technology in the Republic of Korea. Kim shared the first-authorship with Green's graduate student Yu Zhou, who recently defended her Ph.D. "We combined up to 4 such repressor elements in NAND and NOR gates to conditionally stop the production of an output protein. Importantly, these elements can be freely interchanged."

In their optimization of 3WJ Repressor Switches the researchers teamed up with Julius Lucks' group at Northwestern University who had developed "SHAPE-Seq," a method that enables scientists to correlate the function of RNA molecules with their structural states in live cells. Lucks, Ph.D., is Associate Professor of Chemical and Biological Engineering at Northwestern University, Evanston. His group is also independently developing other types of RNA-based repressors using different approaches.

"This study wonderfully illustrates how the Wyss Institute's Molecular Robotics Initiative engages in collaborative activities that cross-disciplinary and institutional barriers to create programmable cellular devices that can move synthetic biology forward to meet real-world challenges," said Wyss Founding Director Donald Ingber, M.D., Ph.D., who is also the Judah Folkman Professor of Vascular Biology at HMS and the Vascular Biology Program at Boston Children's Hospital, as well as Professor of Bioengineering at Harvard's John A. Paulson School of Engineering and Applied Sciences.

More information: De novo-designed translation-repressing riboregulators for multi-input cellular logic, *Nature Chemical Biology* (2019). DOI: 10.1038/s41589-019-0388-1 , nature.com/articles/s41589-019-0388-1

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