Scientists develop electrochemical platform for cell-free synthetic biology

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The new biohybrid system uses non-optical reporter enzymes contained within 16 microlitres of liquid which pair specifically with micropatterned electrodes hosted on a small chip no more than one inch in length. (To be visible, liquid shown here is more than 16 microlitres) Credit: Steve Southon

Scientists at the University of Toronto (U of T) and Arizona State

University (ASU) have developed the first direct gene circuit to electrode interface by combining cell-free synthetic biology with state-of-the-art nanostructured electrodes.

Study results were published today in *Nature Chemistry*.

Long inspired by concepts from the field of electronics, with its circuits and logic gates, synthetic biologists have sought to reprogram biological systems to carry out artificial functions for medical, environmental, and pharmaceutical applications. This new work moves the field of synthetic biology toward biohybrid systems that can take advantage of benefits from each discipline.

"This is the first example of a gene circuit being directly coupled to electrodes, and is an exciting tool for the conversion of biological information into an electronic signal," said Keith Pardee, assistant professor in the Department of Pharmaceutical Sciences at U of T's Leslie Dan Faculty of Pharmacy.

The interdisciplinary effort to create the new system brought together expertise in cell-free synthetic biology from the Pardee lab (U of T), electrochemistry from the Kelley lab (U of T) and sensor design from the Green lab (ASU).

Overcoming practical limits of optical signaling

Pardee, whose research group specializes in developing cell-free diagnostic technologies that can be used safely outside the lab, received widespread attention in 2016 when he and collaborators released a platform for the rapid, portable and low-cost detection of the Zika virus using paper-based synthetic gene networks.

Bringing the capacity to detect the Zika virus outside of the clinic and to

the point-of-need was a crucial step forward, but the approach relied on conventional optical signaling—a change in colour to indicate that the virus had been detected. This posed a challenge for practical implementation in countries like Brazil where viruses with similar symptoms require health care providers to screen for several different pathogens in order to correctly identify the cause of a patient's infection.

This highlighted the need for a portable system that could accommodate many sensors in the same diagnostic test, a capability known as multiplexing. The challenge was that multiplexing with colour-based signaling is not practical.

"Once you get beyond three colour signals, you run out of bandwidth for unambiguous detection. Moving into the electrochemical space gives us significantly more bandwidth for reporting and signalling. We've now shown that distinct electrochemical signals can operate in parallel and without crosstalk, which is a much more promising approach for scaling up," said Pardee.

The new biohybrid system uses non-optical reporter enzymes contained within 16 microlitres of liquid which pair specifically with micropatterned electrodes hosted on a small chip no more than one inch in length. Within this chip, gene-circuit-based sensors monitor the presence of specific nucleic acid sequences, which, when activated, trigger the production of one of a panel of the reporter enzymes. The enzymes then react with reporter DNA sequences that set off an electrochemical response on the electrode sensor chip.

Detecting antibiotic resistance genes

As a proof of concept, the team applied the new approach to detecting colistin antibiotic resistance genes which have recently been identified in livestock globally and represent a serious threat to the use of the

antibiotic as a last resort treatment for infection. Four separate resistance genes were detected, demonstrating the ability of the system to effectively identify and report each gene independently and also in combination.

For synthetic biologists, this new approach represents a potential technical leap forward. Conventional synthetic biology requires that logic calculations be encoded into the DNA of the gene circuit. This can be painstaking, taking months to years to build complex circuits.

"What makes this combined approach so powerful is that the underlying connectivity of the gene circuit sensor outputs can be re-programmed at will by simply modifying the code at the level of the software rather than at the level of the DNA which is much more difficult and time consuming," said Shana Kelley, university professor in the Department of Pharmaceutical Sciences at U of T's Leslie Dan Faculty of Pharmacy, whose research group specializes in the development of highly sensitive electrochemical sensors. Bringing biology-based sensing together with electronic-based logic, memory and response elements, has the potential to transform medicine, biotech, academic research, food safety, and other practical applications, she said.

A powerful toolkit for the future

"This new system enables us to detect many different signals simultaneously, which is essential for diagnostics and monitoring systems," said co-author Alexander A. Green, assistant professor at the Biodesign Institute at Arizona State University. "The electronic output means that in the future it can be readily interfaced technologies like smartphones and distributed sensing arrays that could be brought directly to a patient's bedside."

In Toronto, Pardee and his research group are excited to see where

others in the synthetic biology field will take the system. "We've essentially created a new set of tools and opened up a new venue for signaling. Synthetic biology applications are limited at the reporting step and this has been a significant challenge. With this new combined approach, we think we can really accelerate the field and its capacity to improve lives."

More information: A multiplexed, electrochemical interface for gene-circuit-based sensors, *Nature Chemistry* (2019). DOI: 10.1038/s41557-019-0366-y , nature.com/articles/s41557-019-0366-y

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