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RPPR Final Report
as of 10-Nov-2021

Agency Code: 21XD

Proposal Number: 70039LSRIP **Agreement Number: W911NF-17-1-0143**
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Report Date: 16-Jul-2018 Date Received: 08-Nov-2021
Final Report for Period Beginning 17-Apr-2017 and Ending 16-Apr-2018
Title: A Robotic Laboratory Workstation to Expand the Capabilities of a Toxin Biosensor
Begin Performance Period: 17-Apr-2017 **End Performance Period:** 16-Apr-2018
Report Term: 0-Other

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Distribution Statement: 1-Approved for public release; distribution is unlimited.

STEM Degrees: **STEM Participants:**

Major Goals: We propose to purchase a state-of-the-art laboratory automation workstation to perform high throughput engineering of synthetic microbial strains to vastly expand the capabilities of a DoDfunded biosensor. The additional purchase of an inverted microscope will enable us to rapidly screen the fluorescent responses of these new strains to a large range of target toxins. These instruments will support DoD sponsored research efforts by several labs at UCSD involved in biosensor development, will accelerate progress on a DoD Multidisciplinary University Research Initiative (MURI) related to dynamic artificial cells, and will greatly strengthen and facilitate the education of students with respect to both projects. The biosensor platform that we are currently developing for the DoD will house 2,000 strains of S. cerevisiae and E. coli and will detect many toxins of concern for national security. The current design will make use of existing GFP libraries in these two species, in which each gene is paired with a GFP reporter. These strains will be patterned on a microfluidic device, and the pattern of responses from the fluorescent strains will be used to identify the presence and concentration of target toxins. While this approach will likely be successful in detecting many agents of interest, an expanded effort to include other species in which such libraries do not exist will vastly increase the number and types of water-borne agents that we can detect. In order to transform and characterize the response of new species, we will require a robotic laboratory workstation with the ability to perform high-throughput liquid handling in a highly customizable fashion along with a dedicated inverted microscope to screen their performance. The proposed system will accelerate current DoD supported research programs while providing students and postdoctoral researchers with an opportunity to obtain highly valuable education related to working with state-of-the-art laboratory technology.

The Hasty lab, supported by a DARPA grant (HR011-15-2-0046), aimed at developing a 2,000 strain biosensor chip utilizing yeast and E. coli to detect water toxins of concern to national security. This grant has a $1.8 million budget split evenly across two years. A second source of support for the proposed research activities will be the DoD ARO MURI on dynamic artificial cells (W911NF-13-1-0383). The total budget for this program is $6.25 million for 60 months: the base period budget is $3.75 million and the option budget is $2.5 million. This grant will support work related to studies on monitoring and controlling the dynamics of phospholipid membrane growth, protein modification of membranes, and the function of synthetic circuits within membranes.

Accomplishments: With this grant, we were able to purchase the Biomek FXP Laboratory Automation Workstation and the Nikon ECLIPSE Ti Microscope. This allowed us to accomplish goals on the grants listed below.

The instrumentation significantly enhanced the research and research-related education on two currently funded DoD programs. Program 1: “An online biosensor for the protection of water supplies” (DARPA , HR011-15-2-0046)

and Program 2: “Dynamic Artificial Cells Composed of Synthetic Bioorthogonal Membranes” (Army Research Office (ARO), W911NF-13-1-0383). This new system of equipment for automated strain engineering and screening also provided an excellent opportunity to educate and train several graduate students and postdoctoral fellows on advanced laboratory techniques.

In addition to the development and testing of engineered strains for the detection of more complex contaminants of concern, we have extended our sensing capabilities by scaling up our platform. We integrated a recent Tabor lab library of over 500 uncharacterized bacterial two-component histidine kinase signal transduction systems (TCS) with the multiplexed microfluidic platform to develop a novel synthetic olfactory approach for sensing chemical and biological COCs for which there are no currently available sensors.

(a). MURI project NF-13-1-0383 "Dynamic Artificial Cells Composed of Synthetic Bioorthogonal Membranes" (2013-2019)

We have been developing and optimizing high-throughput microfluidic technologies for in vitro gene circuit design and testing. First, we developed a microfluidic chip that can sustain prolonged (tens of hours) transcription/translation reactions using only 10-20 ul of reagents. A central reaction chamber contains all components for the gene circuit reaction, including DNA, lysate, and premix. Adjacent reservoirs contain lysate and premix, and they diffusively exchange fresh reagents with the reaction chamber through low-height feeder channels. We have iterated through several designs and loading procedures to improve reproducibility, signal-to-noise, and ease of use. The new chip design includes separate chambers that can be loaded simply and sequentially with a pipet tip. Surface tension prevents liquid from moving from low-height to higher regions of the chip until all chambers are loaded with liquid, removing liquid-air interfaces. We plan to use our robotic workstation to massively parallelize this design and allow simultaneous testing of a library of gene circuits in vitro in a suitably modified version of our previously developed dynOMICS chip.

Second, we are developing a technique to create lysate directly on a chip, using bacteria that are programmed to burst open upon detecting the chemical signal AHL. We have shown that this autolysate can support expression from an extracellular plasmid, and we have again tested multiple iterations of chip design to improve its functionality, reproducibility, and ease of loading. The most challenging aspect of the autolysate chip has been reproducibly loading cells into the reaction chambers off the side of the main channel. Again taking advantage of our robotic workstation, we are moving toward a design with multiple reaction chambers in-line with the loading channel, which should significantly improve loading.

(b). DARPA (HR0011-15-2-0046) “An online biosensor for the protection of water supplies”:
We have previously obtained whole genome RNA-Seq data to find transcripts that are correlated with a synthetic, intracellular oscillator circuit. RNA-Seq data was analyzed for highly correlated transcript dynamics between the circuit and the genome using a standard Pearson correlation as well as for highly predictive genes using Sparse Partial Least Squares (SPLS) regression. Interestingly, this analysis led to a small number of functional groups that respond with similar dynamics. The main groups are chaperones, proteases, and tRNAs. While this data revealed interesting relationships between host gene expression and oscillator dynamics, obtaining RNA samples with a high enough time resolution to compare circuit dynamics and host- genome response is challenging. Towards this goal, we've been using high-throughput microfluidic platform, dynOMICS, to characterize the host transcriptome and proteome response to the SLC. dynOMICS allows as many as 4000 unique strains of E. coli to be grown in a continuous culture environment, while a fluorescent reporter for each strain is monitored. This platform allows measurements to be taken every few minutes, far exceeding the temporal resolution obtainable with RNA-Seq. We have previously used this technology to measure the E. coli whole genome response to heavy metal stress as well as transitions between growth on different carbon sources.

(c) ONR: We have developed a novel biosensing technology by integrating newly-developed fluorescent biosensing bacteria with multi-strain, high-throughput microfluidics. The resulting platform can be used to continuously test seawater samples for the presence of contaminants of concern to the health of Navy divers. We have implemented genetically-encoded sense-and-respond systems from various bacterial species in E. coli for the detection of contaminants of concern (mainly organics and heavy metals). Heavy metal-sensing strains were engineered in the Hasty lab, while monoaromatic-sensing strains were designed and initially tested in the Jeff Tabor lab using batch culture techniques. Functional strains were transferred to the Hasty lab for testing in multiplexed microfluidic devices, a technology adaptable to continuous and in situ monitoring. An acoustic droplet ejection robot was used to load the strains in the microfluidic device. The sensor strains grew on-chip in seawater-containing media and

were periodically exposed to inducers of interest by spiking the growth media. Exposure to contaminants of concern produces a specific and dynamic change in the fluorescence signal of the strains. Usually, each strain responds to one or a small subset of inducers, allowing for differentiation of input signals. Further computational analysis of the time series produced by the strains is performed to single out features of interest for prediction purposes. The microfluidic devices that contain the cells are housed in a deployable sensor box that provides a temperature-controlled environment for the bacteria and possesses the necessary hardware and software to continuously capture optical signals from the multiple sensor strains. For this project, the sensor box was further adapted to the specificities of seawater-sample monitoring and the nature of the contaminants we aimed to detect.

Training Opportunities: Nothing to Report

Results Dissemination: Nothing to Report

Honors and Awards: Nothing to Report

Protocol Activity Status:

Technology Transfer: Nothing to Report

PARTICIPANTS:

Participant Type: PD/PI
Participant: Jeff Hasty
Person Months Worked: 1.00 **Funding Support:**
Project Contribution:
National Academy Member: N

Partners

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I certify that the information in the report is complete and accurate:
Signature: Gissel Cortes
Signature Date: 11/8/21 2:53PM

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