

## Rapid Design and Engineering of Smart and Secure Microbiological Systems

Gyorgy Babnigg,<sup>1</sup> Fatima Foflonker,<sup>2</sup> Michael Fonstein,<sup>1</sup> Sara Forrester,<sup>1</sup> Stephanie Greenwald,<sup>1</sup> Christopher S. Henry,<sup>2</sup> Peter E. Larsen,<sup>1</sup> Filipe Liu,<sup>2</sup> Carla M. Mann,<sup>2</sup> Sarah Owens,<sup>1</sup> Arvind Ramanathan,<sup>2</sup> Rebecca Weinberg,<sup>1</sup> Marie-Francoise Gros,<sup>1,3</sup> Philippe Noirot,<sup>1,3</sup> Tomoya Honda,<sup>4,5</sup> Yasuo Yoshikuni,<sup>4,5</sup> Steven R. Fleming,<sup>6</sup> Ashty S. Karim,<sup>6</sup> Brenda Wang,<sup>6</sup> Michael C. Jewett,<sup>6</sup> Mark Mimeo,<sup>7</sup> and **Dionysios A. Antonopoulos**<sup>1\*</sup> (dion@anl.gov)

<sup>1</sup>Biosciences Division, Argonne National Laboratory, Lemont, IL; <sup>2</sup>Data Science and Learning Division, Argonne National Laboratory, Lemont, IL; <sup>3</sup>National Research Institute for Agriculture, Food and Environment (INRAE), France; <sup>4</sup>Lawrence Berkeley National Laboratory, Berkeley, CA; <sup>5</sup>Joint Genome Institute, Berkeley, CA; <sup>6</sup>Northwestern University, Evanston, IL; <sup>7</sup>University of Chicago, Chicago, IL

**Project Goals: The long-term goal for this Project is to realize secure biodesign strategies for microbial systems that operate in the dynamic abiotic and biotic conditions of natural environments, thus enabling systems-level and rational biological design for field use. There are several key challenges to incorporating safeguard systems at the design stage including: (1) lack of knowledge for how well safeguards operate across the broad set of environmental and physiological conditions that an organism experiences; (2) a need to integrate the safeguard with other cellular components so that it can sense and recognize specific signals from the intracellular or extracellular environment, and mediate a response; and (3) a need for rapid and reliable methods to engineer and optimize the biological components for safeguard construction and functional integration. To address these challenges, we propose to utilize recent advances in the fields of synthetic biology, artificial intelligence (AI), and automation, which are creating the conditions for a paradigm shift in our understanding of the ways that cellular function can be designed at the level of bacterial communities.**

The design and application of successfully engineered biosystems requires an understanding of how engineered microbes will interact with other organisms – either as one-on-one competitors, for example, or in the context of microbial consortia. Engineering microorganisms from first principles for non-laboratory, environmental applications is inherently challenging because: (1) engineered systems tend to quickly revert back to their wild-type behaviors; and (2) these systems typically pay a price in reduced fitness making them uncompetitive against invasive contaminating species (i.e., metabolic burden). A key question is how do sensing, signaling, and metabolism contribute to the stabilization and destabilization of these interactions? Here, the organization, control, stabilization, and destabilization of natural and engineered microbes will be investigated through a synthetic biology approach. The approach will enable development of (1) single-strain systems capable of detecting and responding to target organisms in the environment; (2) a pipeline for refining and engineering biological constructs in new non-model host organisms; and (3) improved systems for the rapid designing, engineering, and assaying of new biological modules. This coupled approach to design and build safeguard systems for intrinsic biocontainment that are predictable and portable across bacterial species, would focus on microbes that are part of the beneficial plant microbiome. A long-term goal beyond the proposed research is to enable the engineering of communities of microorganisms

based on first principles of biological design that mimic the smart performance of microorganisms observed in natural systems. This will enable a new vision of biosecurity and biocontainment that harnesses the underlying mechanisms of resource management occurring within and between organisms.

*This Project is funded by the Biological Systems Science Division's Genomic Science Program, within the U.S Department of Energy, Office of Science, Biological and Environmental Research.*