Trustworthy Synthetic Biology: Plant-Based Biosensing

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ABSTRACT

After establishing the four principles of Trustworthy Synthetic Biology—safety, assuredness, efficiency, and robustness—a team of researchers at the Johns Hopkins University Applied Physics Laboratory (APL) generated trustworthy plant sensor and reporter systems. Their work, initially funded as an APL independent research and development project, has since transitioned to a sponsor-funded project.

The field of synthetic biology combines the disciplines of engineering and biology to construct novel biological parts, molecular devices, and even entire organisms. It has the potential to change the world. With application spaces from health care, to agriculture, to biofuel production, to materials, one may wonder if there is anything that cannot be done with synthetic biology.[1]

While synthetic biology holds great promise, many challenges remain. Living systems are infinitely complex and difficult to manipulate. Synthetic biologists have mastered techniques for building and introducing engineered components into a variety of living systems, but these components do not always perform predictably. Introduction of engineered components often has negative impacts on the host organism, and genetically modified organisms are often not as robust as their wild counterparts. The cost associated with genetic engineering and the unknown impact of these modified organisms on environmental or human health limit their potential for use beyond the lab. For the full potential of synthetic biology to be realized, these challenges must be addressed.

To be deployed on a wide scale, synthetic biology products must perform their specified task when they are supposed to and for as long as they are supposed to, under operationally relevant conditions. These characteristics are reminiscent of the principles of Trustworthy Computing that were established nearly 20 years ago by Microsoft and include reliability, security, privacy, and business integrity. Parallels are often drawn between the genetic programming of organisms and that of computer software and hardware, with security being key for both. We envision a similar paradigm of Trustworthy Synthetic Biology (TwSB).

To provide the basis for this strategic independent research and development effort, we proposed four principles of TwSB: safety, assuredness, efficiency, and robustness. Safety refers to intrinsic biocontainment mechanisms that cannot be mutated or degraded over time, preventing escape and interbreeding of the genetically modified population with the wild population. Assuredness refers to the reliability and stability of engineered components; that is, they must work as planned in the intended environment. Efficiency refers to working with an organism's intrinsic biology instead of trying to override it. This is necessary to minimize negative impacts on fitness. Robustness refers to generating genetic diversity among modified species while leveraging population-level responses for signal amplification.

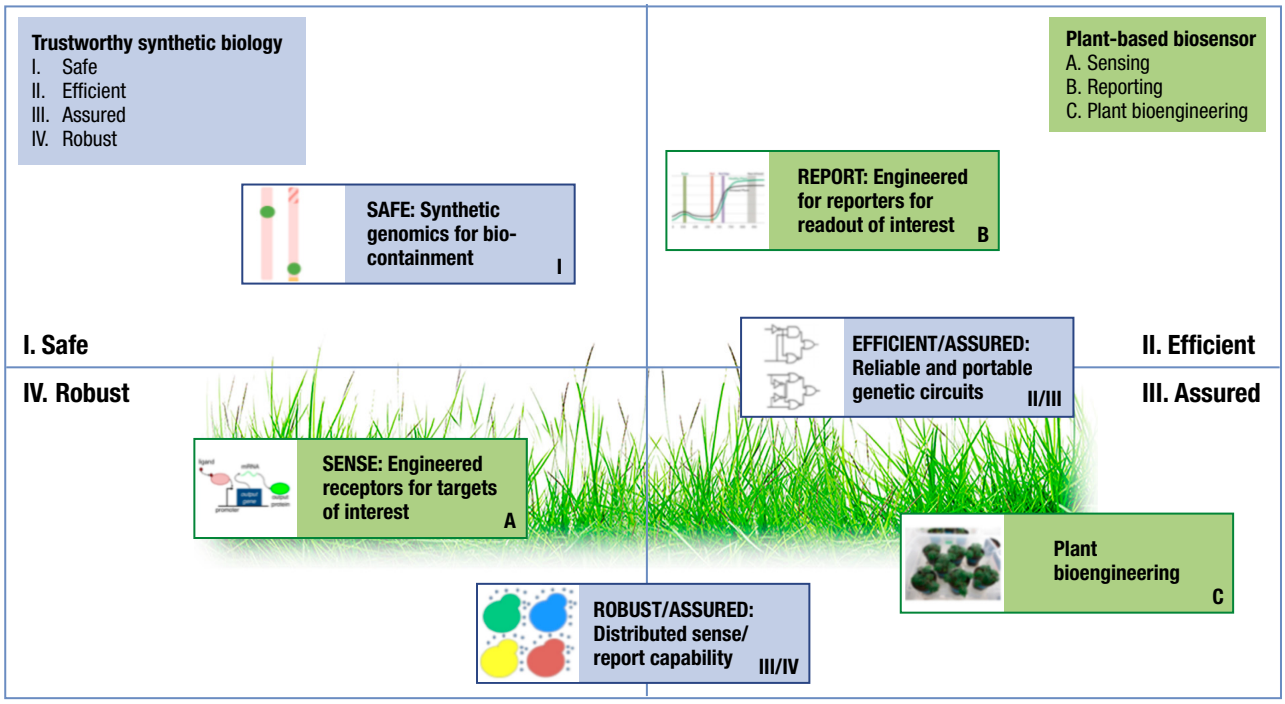

Figure 1. Overview of the TwSB project. Tasks aligned with principles are in blue boxes. Application-specific tasks are in green boxes.

With these TwSB principles in mind, we set out to generate a deployable plant-based sensor (Figure 1). Our first set of aims was to tackle issues related to biocontainment, genetic programming, and signal amplification. Our second set of aims was to realize specific applications of sensing and reporting and plant transgenesis. Much of the work on this project laid the groundwork for compelling and impactful sponsor-funded research.

First, we sought to develop a general strategy for stable biocontainment of genetically modified organisms to ensure safety of our proposed technology. Our initial strategy was to employ CRISPR-Cas9 to induce large-scale genome restructuring in such a way that maintained and preserved mating among modified organisms while prohibiting mating with the wild population. As a proof of concept, work was done in a genetically tractable microbial system, the baker's yeast *Saccharomyces cerevisiae*. However, concurrently, others in the field demonstrated the need for far more complex rearrangements for total genetic isolation,[2] achieved only with a strong negative impact on the fitness of the yeast.

Next, focusing on genetic programming, we addressed the TwSB principles of assuredness and efficiency. Naturally occurring gene circuits commonly break. The goal of this task was to develop reliable and portable genetic circuits. We sought to develop general methods for stabilizing gene circuits based on modifying the intrinsic properties of the gene circuit to prevent mutation and subsequent malfunction. We based our experiments on a microbial system with a previously characterized genetic circuit, using an existing circuit that limits population growth via expression of a toxin when the population reaches a certain density.[3] As anticipated, reproducible failure of the circuit was observed; however, we were unable to identify causative mutations via targeted sequencing of the circuit itself. These results indicate that the gene circuit was not accumulating mutations and malfunctioning; rather, the host was evolving to overcome the growth restrictions imposed by the population control circuit. Results highlight complexities of engineering in a living system and illustrate the importance of working with an organism's intrinsic biology to develop reliable genetic circuitry.

Finally, robustness was addressed through a signal propagation approach. In this task, we sought to increase population performance via the development of enhanced signaling capabilities. To this end, we designed and engineered a portable synthetic extracellular signaling pathway that allows for widespread dissemination of a signal through a population. The pathway we developed uses the volatile organic compound, diacetyl, a known diacetyl receptor from the nematode *Caenorhabditis elegans*, ODR-10, and the downstream signaling components from the baker's yeast mating factor pathway.[4] For an initial proof of concept, this pathway was engineered in yeast, but it was designed to be portable to algae and even plants. We engineered two strains of yeast, one capable of generating the diacetyl signal (the sender) and the other capable of responding to it (the receiver), and we were able to demonstrate function of these components. Employing this pathway in conjunction with molecule-specific sensing components could

provide increased sensitivity for the molecule being detected. Much of the work transitioned to a sponsor-funded research effort, and we have begun prototyping key components in plants.

With principles defined, we set out to generate TwSB-compliant plant sensor and reporter systems. To demonstrate a plant sensor, we chose microcystin, a globally distributed environmental toxin produced by harmful algal blooms, as our analyte.[5] We used computational protein design to generate a novel protein receptor as the sensor recognition element and constructed an engineered transcription factor that would be stabilized in the presence of microcystin, binding to DNA and activating the transcription of a reporter. This approach had limited success, and we investigated bacterial histidine kinase receptors (HKRs) as an alternative platform for engineering novel sensing domains for the detection of microcystin.[6]

In addition to engineering sensors for microcystin detection, we worked to generate novel reporters for standoff detection in plants. Currently, alterations in pigment production are primarily used for standoff detection, since that is the easiest way to alter plant reflectance; however, modifying pigment content often has negative impacts on plant fitness and is not covert. As an alternative to existing reporters, we pursued the development of hyperspectral reporters for plants. All organisms capable of photosynthesis produce two types of chlorophyll, *a* (λmax = 665 nm) and *b* (λmax = 652 nm). In addition to these chlorophylls, algae produce a number of additional types of chlorophyll. Among these is chlorophyll *f* (λmax = 705 nm), which can be produced from chlorophyll *a* via expression of a single enzyme, chlorophyll *f* synthase.[7] To test the feasibility of using chlorophyll *f* as a hyperspectral reporter, we performed proof-of-principle imaging experiments. Our studies indicated that conversion of as little as 1% of chlorophyll *a* to chlorophyll *f* would be detectable by hyperspectral imaging in a relevant organism at standoff distances.

In summary, the TwSB independent research and development effort provided a platform for us to establish the principles of TwSB: safety, assuredness, efficiency, and robustness. Further, it enabled us to develop key concepts for a Defense Advanced Projects Agency (DARPA) performer role. Beyond that, this work positions APL to become a leader in the development and deployment of living biological sensors.

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