ACS Synthetic Biology
Editorial
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Engineered Microbes for Therapeutic Applications

As synthetic biologists, we share a focus on the construction of new genetic elements or circuitry in living cells, whether at the level of large-scale synthesis efforts or the forward engineering of relatively small networks. We also enjoy a shared luxury when presenting our work to biologists; the complexities associated with the physiology of a host organism are typically a secondary concern. The goal is to create something new. While such a perspective is liberating in terms of the dogmas that are often associated with niche groups in science, we as synthetic biologists also acknowledge the responsibility that comes with the engineering approach. We must endeavor to engineer genetic elements or circuits that have the potential for useful applications. In this regard, perhaps the popularity of the field stems, in part, from its natural position at the interface of basic research in academia and product development in industry. In this issue, we highlight methods and approaches that will help to catalyze the transition from basic science to applications.

Many of the genetic circuit applications to date have employed the use of plasmids. For synthetic biology, self-replicating plasmids possess at least two significant advantages over integration into the host genome. They can often be synthesized at a high copy number, which can even be made tunable, and they often reside and replicate outside the milieu of the host nucleoid. These properties can significantly increase the signal while at the same time decreasing noise that can arise from small numbers of reactant biochemical species. However, there are limitations for large plasmid-based designs, such as the often increased difficulty in using PCR due to long and/or repeating sequences, fewer available unique restriction sites, and lower transformation efficiencies. Multiple smaller plasmid types can be employed, but this necessitates the use of multiple selective markers, which can be expensive and toxic to the host. In the first article of this issue, Schmidt *et al.* demonstrate a method for the stable maintenance of multiple plasmids within a single cell. They show how small multiple-plasmid systems can be scaled up without the need for multiple resistance markers, which enables the rapid prototyping of increasingly complex circuits that possess a high signal-to-noise ratio.

Genetic sensors are a ripe area for applications in synthetic biology. This is perhaps natural given that our classical understanding of gene regulation has arisen from the remarkable sensing abilities of viruses such as lambda phage. In this context, the original toggle switch served to christen an era where genetic circuitry is engineered to detect an intracellular signal and then flip a switch in response to a defined input. While there have been numerous variations on this general theme over the years, there have been few attempts to decouple the basic logic of sensing and switching. Archer *et al.* describe a novel system whereby sensing is achieved through the use of a recombinase that can be programmed to turn on gene expression in the presence of an upstream signal. Specifically, they demonstrate how the gut inflammatory signal nitric oxide can be used to trigger a permanent and heritable change in gene expression via a recombination event. The authors discuss how the system may one day be useful in the treatment of inflammatory bowel disease. Importantly, their sensing method is quite general; any inducible promoter can be used to trigger an irreversible recombination event. Thus the method provides a new modular tool in the development of genetic sensors.

To date, many of the genetic circuits designs have relied on *E. coli* as the "chassis" on which to engineer genetic circuits. In moving toward applications, it is becoming increasingly clear that other microbes are often easier to adapt to clinical use. One straightforward reason is the FDA regulatory process; any substance introduced into a human via the bloodstream has to be regulated as a drug. So if a bacterium is engineered to propagate through the bloodstream and into tumors, significant resources must be invested in order to demonstrate safety. Such an investment can exceed $1B and this barrier is often the point at which interesting therapeutic possibilities stall. Thus, an important question is the degree to which existing circuits can be adapted to new hosts that are already used in a clinical setting. Prindle *et al.* explore genetic circuits in *Salmonella typhimurium*, a therapeutically relevant microbe with safety precedence in human clinical trials for cancer therapy. They find that the core functionality of several classical circuits is preserved without any plasmid modification. Using these dynamic networks, they measured strain-specific differences in protein degradation and gene expression rates. Computational modeling recaptured the subtle differences in circuit function when using these *S. typhimurium*-specific parameters. Future work will involve characterizing more distantly related microbes to define the space of "circuit-ready" hosts in the microbial evolutionary tree, thus enabling a new generation of potential applications for synthetic biology.

A key step in translating synthetic biology will be identifying design criteria for building genetic circuits in application specific contexts. One of the holy grails in the application space is to engineer tumor-killing bacteria that can intelligently sense and deliver therapeutics in tumor environments inside of a host. While there has been considerable effort in using bacteria as cargo-producing delivery vehicles, little effort has been made to scale up the sophistication of bacteria's dynamics sensing and cargo delivery capabilities. One reason is that going beyond traditional genetic engineering approaches requires standardization and model guided-design, which synthetic biology is still developing *in vitro*. As a step toward quantitatively engineering more complex bacteria delivery profiles *in vivo*, Danino *et al.* characterize population dynamics and gene expression of tumor-targeting bacteria *in vivo* using a mouse tumor model. They describe how plasmid-loss, due to loss of antibiotic selection, can generate tunable, transient drug-delivery profiles. The authors developed a mathematical model in which they characterized how strain growth rate, plasmid-loss, and dosage affect drug delivery production

Special Issue: Engineered Microbes for Therapeutic Applications

Received: October 3, 2012
Published: October 19, 2012

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profiles *in vivo*. In the future, combining both mathematical modeling and strain engineering will allow for creation of designer bacteria with different release kinetics to deploy cargo in tumor environments.

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Notes

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