ACS SyntheticBiology

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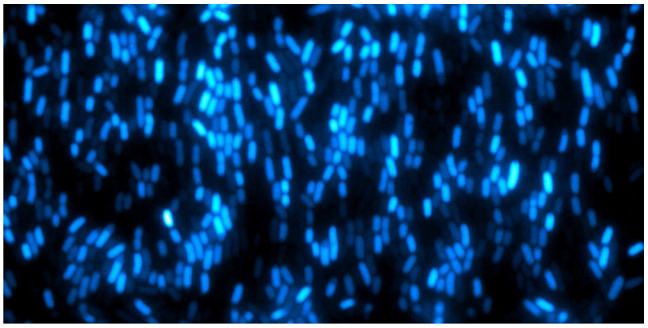
GENETIC CIRCUITS IN SALMONELLA

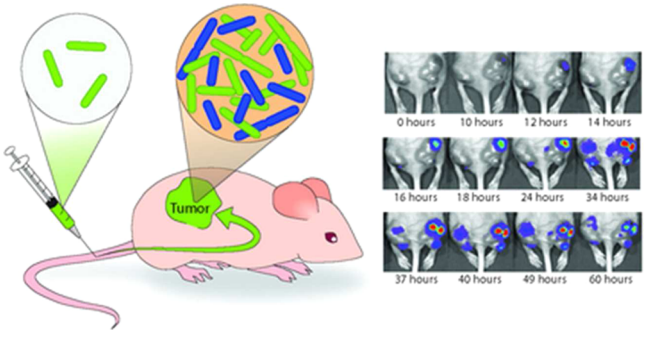
In recent years, there has been growing interest in the application of synthetic biology to medicine. While the field has achieved much success using the genetic circuits approach, the majority of these studies were performed *in vitro* in *E. coli*. Important clinical problems exist *in vivo* and will likely require application-specific hosts due to safety, immunogenicity, and metabolic requirements. Here, Prindle *et al.* (DOI: 10.1021/sb300060e) demonstrate that *Salmonella typhimurium*, a clinically relevant strain with safety precedence in human clinical trials, is capable of executing a variety of published genetic circuits.



Using its simple genetic manipulation tools and built-in advantages specific to cancer, the authors show that *S. tyhimurium* can execute genetic circuits ranging from toggle switches to synchronized oscillators at the colony level. This work supports the application of sophisticated genetic programming to cancer therapy and paves the way for a new generation of synthetic biology in the clinical arena.

GENE EXPRESSION DYNAMICS OF TUMOR-TARGETED BACTERIA

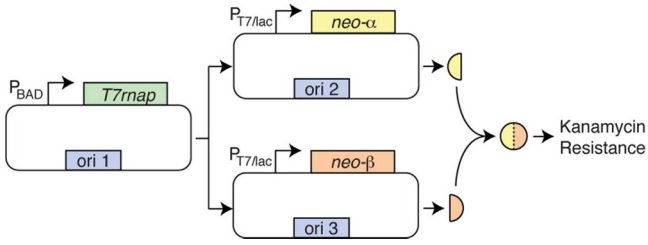
Synthetic biology has recently garnered interest in the field of therapeutic delivery. While previous work has focused on the design criteria of genetic circuits that function *in vitro*, very few studies have explored engineering genetic circuits in an *in vivo* context. The types of delivery strategies described so far lack control in the dosage delivered to tumors and tend to yield exceptionally high dosage due to ongoing bacterial growth, potentially leading to off-target effects. Now, Danino *et al.* (DOI: 10.1021/sb3000639) take advantage of the reality of plasmid loss *in vivo* that occurs due to lack of antibiotic selection, to generate tunable dynamic expression profiles in tumors.



To predict and follow the dynamics of plasmid expression, the authors developed a mathematical model of bacterial growth dynamics and plasmid-based expression for bacterial populations *in vivo*. This is the first quantitative model for bacterial dynamics in tumor models and allows for design of specific drug delivery profiles as well as fine-tuned control with additional layers from genetic circuits.

MAINTENANCE OF MULTIPLE PLASMIDS IN *E. COLI*

The creation of synthetic gene circuits in *E. coli* is most often done with the use of plasmids. The ease with which plasmids can be transformed into *E. coli* and the many tools that are available for manipulating their DNA sequences make them a robust platform for designing, testing, and implementing regulatory architectures. However, as the size and complexity of synthetic gene circuits increase, so does the need for plasmid-based frameworks that can simultaneously maximize the amount of DNA and minimize the strain on the host. Here, Schmidt *et al.* (DOI: 10.1021/sb3000589) develop two new methods for stably maintaining up to three different plasmid types in *E. coli*.



Using an antibiotic as the only selective marker, the authors show not only that these multiplasmid systems can be maintained with hosts for long-term serial dilution experiments but also that they can easily be transformed into almost any strain without the need for special transformation protocols.

ENGINEERED *E. COLI* TO DETECT GUT INFLAMMATION

Recent work has shown that micro-organisms re-engineered to include synthetic gene circuits can perform a variety of useful biological functions. Here, Archer *et al.* (DOI: 10.1021/sb3000595) detail the design and construction of a synthetic gene regulatory circuit in *E. coli* that enables microbial cells to sense nitric oxide, a signal given off by mammalian cells during inflammation.

The authors designed a circuit using a DNA recombinase that ensured robust and permanent cellular response to inflammation, even if cells are exposed to the inflammatory signal only transiently. In addition to extensive characterization *in vitro*, the authors show that the engineered microbial cells can sense nitric oxide produced by mouse ileum explant cultures.

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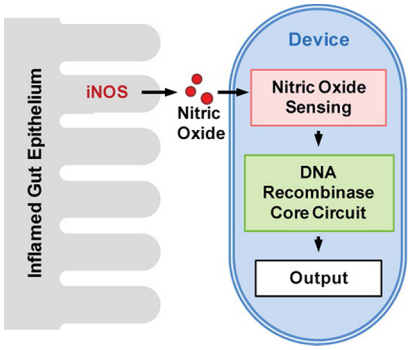
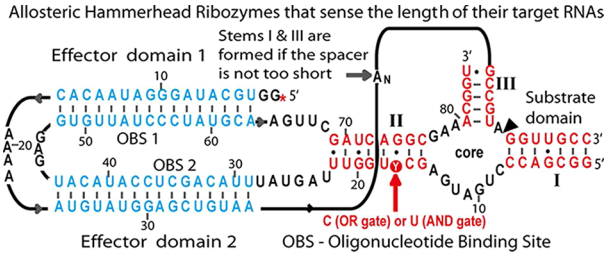


In the future, the synthetic microbial devices described here may not only detect various diseases, but treat them as well using their modular sensory and response components.

INTEGRATED DIGITAL CIRCUITS TO SCALE-UP MOLECULAR COMPUTATION

Although several advances in the engineering of *in vitro* and *in vivo* molecular computing systems have been reported, the engineering of these systems still lacks in comparison to silicon etched technology in terms of functionality, reliability, and complexity. Here, Penchovsky (DOI: 10.1021/sb300053s) describes the computational design and experimental validation of integrated molecular circuits based on single allosteric ribozymes.

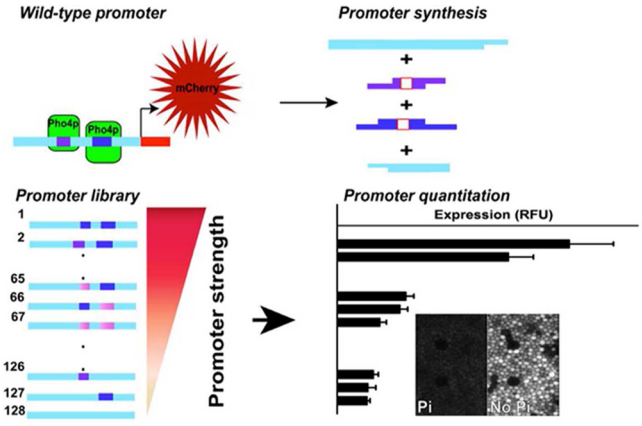


The author demonstrates that a single allosteric ribozyme can be programmed to interact with various signal RNA and DNA molecules, to work as an integrated digital circuit, with the functionality of electronic circuits built from up to five logic gates.

RAPID SYNTHESIS OF EUKARYOTIC PROMOTER LIBRARIES

Transcriptional regulation is one of the most important control mechanisms employed by cells and is conducted primarily through the interplay between transcription factors and genomic DNA elements, the promoters and enhancers. Despite considerable efforts toward deciphering and characterizing these elements, transcriptional regulation is still a poorly understood mechanism. Here Rajkumar and Maerkl (DOI: 10.1021/sb300045j) describe the generation of hundreds of synthetic eukaryotic promoters, each containing defined and precise changes.

In this Tutorial, the authors present a rapid and robust method for the construction of eukaryotic synthetic promoter libraries and their integration into yeast by homologous recombination. This approach promises to help researchers decipher how the cell integrates promoter architecture and gives rise to a specific regulatory output. Fully characterized



promoter libraries will be valuable resources for building and optimizing novel genetic and metabolic networks.

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