ACS SyntheticBiology

In This Issue

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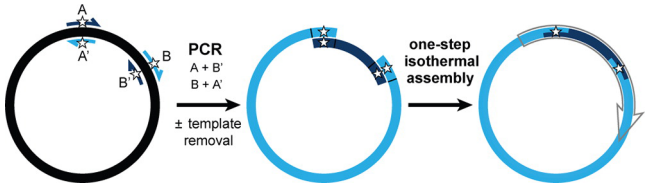
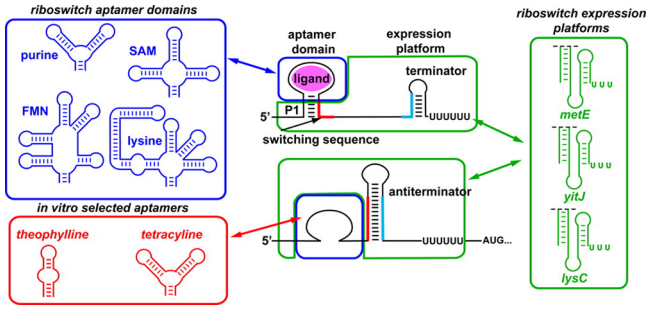
MULTICHANGE ISOTHERMAL MUTAGENESIS



Site-directed mutagenesis (SDM) is one of the most frequently used techniques in molecular biology. Its widespread use to make specific coding changes in proteins has driven fundamental discoveries in the fields of genetics, biology, and biochemistry. Here, Mitchell et al. (DOI: 10.1021/sb300131w) describe Multichange ISOthermal (MISO) mutagenesis, a new technique allowing simultaneous introduction of multiple site-directed mutations into plasmid DNA.

The authors show that MISO mutagenesis is more robust and flexible than commonly used SDM techniques as it (i) enables the introduction of an increased number of base substitutions in a single experiment, (ii) overcomes limitations in plasmid size plus reduces the amount of sequencing following mutagenesis, and (iii) is also capable of introducing deletions and insertions into plasmid DNA. MISO mutagenesis is a powerful approach that could be useful to anyone attempting to introduce DNA modifications into plasmid DNA.

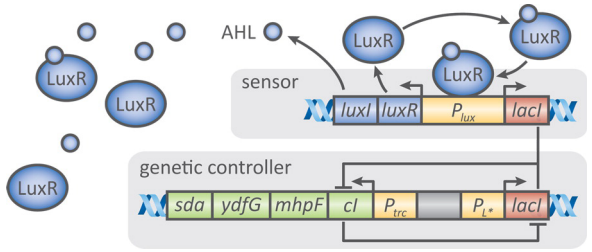
FACILE ENGINEERING OF NOVEL GENETIC REGULATORY DEVICES



RNA receptors (also known as "aptamer domains") are highly modular and can function in the context of a variety of natural and synthetic contexts. However, the challenge has always been to be able to transduce the binding event into a readable signal to create a sensory device, which requires the development of a regulatory domain that is highly specific for the aptamer. Now, Ceres et al. (DOI: 10.1021/sb4000096) report that the regulatory switch of some riboswitches is highly modular and can host a variety of aptamers. Notably, these receptors included small-molecule binding aptamers derived from artificial selection.

To understand why the secondary structural switch can be regulated by non-native aptamers, the authors performed a systematic mutagenic survey of several riboswitches and found strong support for "encoded co-transcriptional folding". They demonstrate that there is no specific relationship between the aptamer domain and expression platform in some riboswitches and that all of the information necessary for the secondary structural switch is encoded in these modular expression platforms. This work represents a significant advance in the understanding of the mechanism of riboswitches.

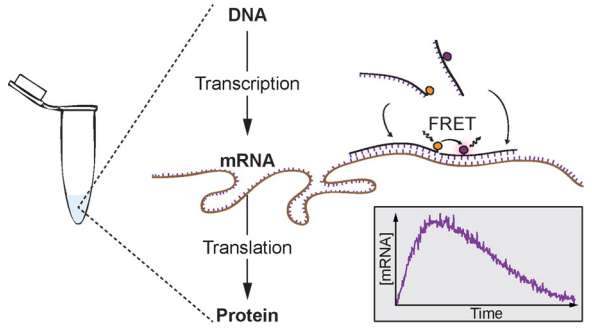
A GENETIC CIRCUIT FOR DYNAMIC METABOLIC ENGINEERING



In metabolic engineering, genetic manipulation strategies for altering the metabolic capabilities of microbes are typically static, often leading to impaired growth and productivity. Dynamic manipulation of gene expression can prove valuable for improving metabolic engineering strategies. Now, Anesiadis et al. (DOI: 10.1021/sb300129j) assess the sensitivity of an integrated *in silico* design, based on a density-sensing module and a genetic toggle switch, to the parameters of the genetic circuit.

On the basis of a serine-producing case study, the authors explore the effects of the genetic circuit parameters on serine production using global sensitivity analysis. They report on the effects of changing three parameters, identified to have the greatest impact on serine production, on the productivity, yield, titer, batch and switching time of the toggle switch. The development of this type of synthetic biology-based strategy for metabolic engineering applications has the potential to make significant improvements to bioprocess productivity and commercialization of poorly growing strains.

REAL-TIME mRNA MEASUREMENT USING BINARY PROBES



In vitro transcription and translation reactions, particularly the PURE system of cell-free protein synthesis, are becoming popular approaches to *in vitro* synthetic biology. However, a more quantitative understanding of transcription and mRNA dynamics will help to rationally engineer these systems. Here,

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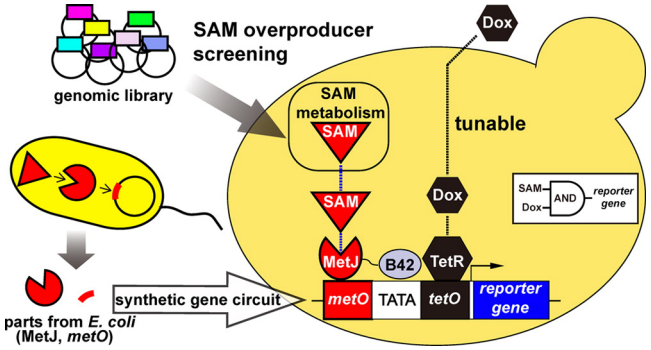
Niederholtmeyer et al. (DOI: 10.1021/sb300104f) use binary probes to measure mRNA concentration during these reactions.

Binary probes are two DNA oligonucleotides that each carry a fluorophore and that are designed to bind to a sequence on the mRNA. The authors designed and tested different probe-target pairs and found that the position and sequence environment of the target site is important for binding kinetics and signal. They then applied the best-performing design to measure mRNA dynamics and repression of mRNA synthesis by the TetR transcription factor in a commercial cell-free protein synthesis kit.

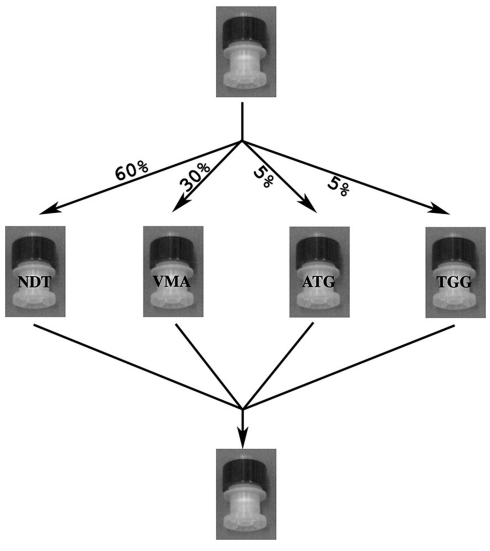
SYNTHETIC GENE CIRCUIT-MEDIATED MONITORING OF ENDOGENOUS METABOLITES



Monitoring levels of metabolites in living cells is essential to industrial and pharmaceutical research. While this is typically performed using a fluorescent reporter gene in conjunction with an endogenous promoter responsive to the metabolite of interest, a synthetic gene circuit can be used when such a promoter is not available. Here, Umeyama et al. (DOI: 10.1021/sb300115n) use the met operator and MetJ repressor of *E. coli* to construct a synthetic gene circuit that specifically responds to a key metabolite, *S*-adenosylmethionine (SAM), in *Saccharomyces cerevisiae*.

The authors constructed a single and dual-input circuit, responsive to SAM levels, and screened a genomic library, successfully identifying GAL11 as a novel multicopy enhancer of SAM levels. This work demonstrates the potential and utility of synthetic gene circuit-mediated monitoring of metabolites.

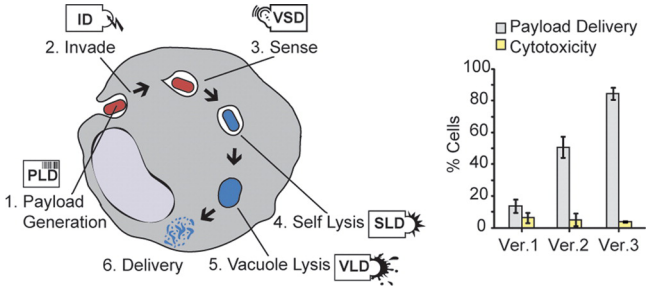
ELIMINATING REDUNDANT AND STOP CODONS



Existing strategies for the elimination of redundant and stop codons during the chemical synthesis of degenerate oligonucleotides includes the use of an expensive cocktail of 20 trimer-phosphoramidites. Here, Gaytán and Roldan-Salgado (DOI: 10.1021/sb3001326) describe a less-expensive strategy using standard monomer-phosphoramidites and a simplified resin-splitting procedure.

This method also yielded new interesting fluorescent proteins when it was tested on the synthetic gene coding for the far-red fluorescent protein mKate, suggesting that this approach could be useful for improving the properties of enzymes and antibodies with potential industrial and clinical applications.

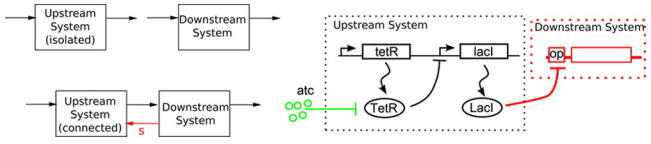
MODULAR DESIGN OF A SYNTHETIC PAYLOAD DELIVERY DEVICE



Synthetic biology is premised on the notion that human-designed biological function can be 'built-up' according to a progression of functional abstractions much the way software is written. Here, Huh et al. (DOI: 10.1021/sb300107h) examine this premise in the context of a problem-driven design specification requiring three levels of abstraction and five distinct functional modules.

The authors challenge existing engineering toolkits to identify the bottlenecks, limitations, and general feasibility of designing complex genetic systems. They describe a synthetic payload delivery device which enables a bacterium to invade cancer cells, sense the vacuole microenvironment, self-lyse, and then deliver a 'payload' to the cytoplasm. Though trouble-shooting and optimization are still required to engineer an efficient system, such a simplification of the design process suggests an increase in the complexity of engineered genetic systems.

RETROACTIVITY CONTROLS THE TEMPORAL DYNAMICS OF GENE TRANSCRIPTION



The temporal dynamics of gene transcription cover a central role in a cell's ability to sense and respond to time-varying input stimuli and are at the basis of a number of synthetic circuits. Hence, a number of studies have been focusing on uncovering the molecular mechanisms that control the dynamics of gene expression. Now, Jayanthi et al. (DOI: 10.1021/sb300098w) provide a combined theoretical/experimental study to characterize the effects of retroactivity on the temporal dynamics of gene transcription.

The authors show that even when the effects of retroactivity on the steady state behavior of a transcription component are

modest, the dynamic effects are dramatic. They also provide mathematical formulas that predict the extent of these effects based on measurable parameters. These ultimately provide a tool to predict the dynamic behavior of connected systems even in the absence of modularity, while suggesting a new mechanism for tuning the temporal dynamics of gene transcription.

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