ACS SyntheticBiology

In This Issue

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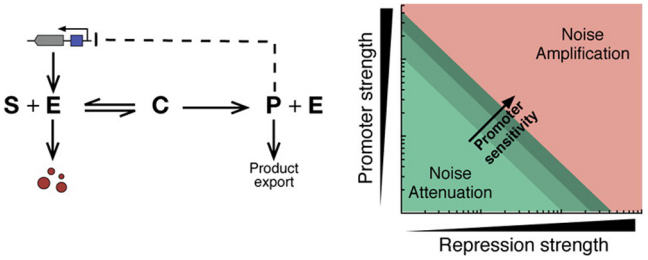
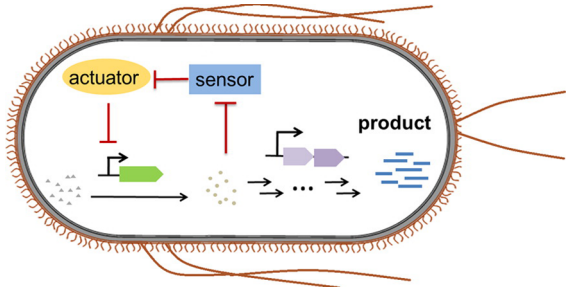
NOISE PROPAGATION IN SYNTHETIC GENE CIRCUITS FOR METABOLIC CONTROL



Biochemical noise causes isogenic cells to reach different enzyme expression levels, potentially translating into large flux variability across cell cultures. Despite these considerations, the effect of noise remains overlooked in the design of genetic circuits for metabolic engineering. Here, Oyarzún *et al.* (DOI: 10.1021/sb400126a) use a model-based approach to quantify the effect of circuit parameters on biochemical noise, ultimately proposing new design criteria that allow for noise attenuation.

The authors use a stochastic model for a negative genetic feedback circuit, parametrized in terms of the input-output characteristic of the promoter. They found that metabolic noise, the fluctuations in the number of product molecules as measured by the squared coefficient of variation of its stationary distribution, can be amplified or attenuated with respect to the strength of the feedback repression. They also show that the ability of the circuit to attenuate noise is subject to a trade-off between two design parameters: the promoter strength and the repression strength. Thus, noise attenuation can be achieved by either a strong promoter under weak repression or a weak promoter under strong repression.

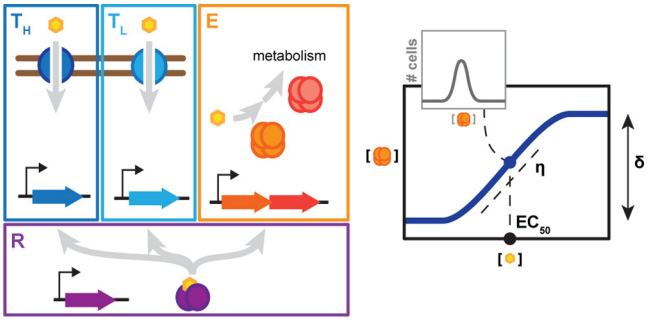
NEGATIVE FEEDBACK REGULATION OF FATTY ACID PRODUCTION



The ability to engineer metabolic biosynthetic pathways has allowed for the microbial production of several useful chemicals. While metabolic imbalances often limit productivity and yield, synthetic regulatory circuits have been shown to be able to balance engineered pathways and, thus, increase productivity. In this paper, Liu *et al.* (DOI: 10.1021/sb400158w) describe the development of a negative feedback regulatory circuit based on a malonyl-CoA-based sensor-actuator.

The first step in fatty acid biosynthesis, which is also the rate-limiting step, is the synthesis of malonyl-CoA. In *E. coli*, malonyl-CoA is biosynthesized from acetyl-CoA by acetyl-CoA carboxylase encoded by *acc*ABCD (*acc*). In this study, the authors show that a malonyl-CoA-based negative control system can alleviate the toxicity caused by *acc* overexpression. Application of this system to the fatty acid pathway also improved fatty acid titer and productivity by 34% and 33%, respectively.

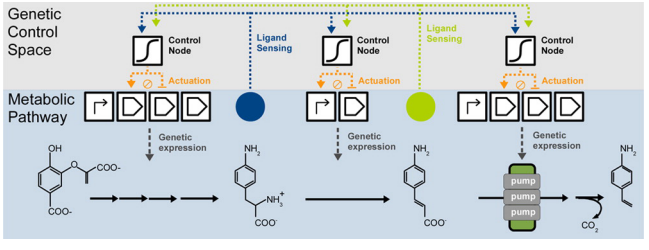
TRADE-OFFS IN ENGINEERING SUGAR UTILIZATION PATHWAYS



Titratable systems are powerful tools for programming genetic circuits and optimizing enzyme levels in metabolic pathways. Native sugar utilization pathways offer built-in titratable systems, although these pathways can exhibit undesirable responses such as bimodality or switch-like behavior. Here, Afroz *et al.* (DOI: 10.1021/sb400162z) apply mathematical modeling and single cell analyses of the L-arabinose utilization pathway in *E. coli* to investigate how alterations to these pathways influence the desirability of the response.

The authors found that pathway alterations, such as constitutively expressing transporters or disrupting catabolism, come with inherent trade-offs. For example, removing catabolism reduces the required amount of inducer at the cost of a sharper and less uniform response. Within these alterations, the authors also found that a single alteration—constitutively expressing the high-affinity transporter—could readily generate a uniform response. Overall, the findings reported here indicate that there is no perfect set of alterations to achieve an ideal titratable response and suggest general design principles when engineering sugar utilization pathways in microorganisms.

DESIGNING RNA-BASED GENETIC CONTROL SYSTEMS



Special Issue: Circuits in Metabolic Engineering

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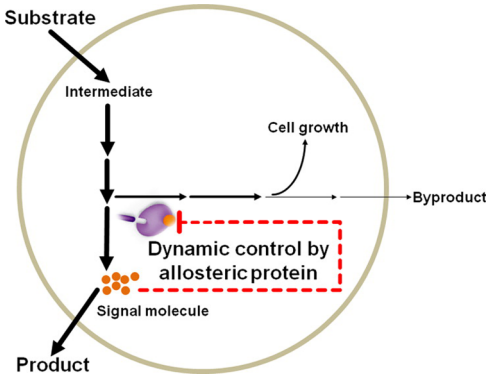
Engineered metabolic pathways can be augmented with dynamic regulatory controllers to increase production titers by minimizing toxicity and helping cells maintain homeostasis. Here, Stevens and Carothers (DOI: 10.1021/sb400201u) use deterministic models of regulation of a metabolic pathway engineered to produce *p*-aminostyrene (p-AF), to computationally study the effect of different types of regulation on the overall pathway production.

To computationally assess the viability of designing a control system to optimize titers and maintain cell viability, the authors analyzed and performed Monte Carlo simulations of 728 unique RNA-based dynamic genetic control system designs. Results show general pathway-specific trends in successful control architectures and identify control topologies that are most consistent with higher production yields. Additionally, by filtering parameter inputs by production outputs, the authors show that highly performing topology implementations can be constructed from RNA components that can be readily generated in the laboratory.

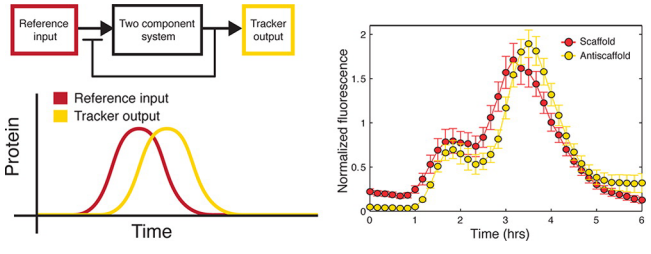
RATIONAL DESIGN OF ALLOSTERIC REGULATION OF HOMOSERINE DEHYDROGENASE



One of the challenges of metabolic engineering and synthetic biology is the precise control of cellular metabolism. Here, Chen *et al.* (DOI: 10.1021/sb400133g) use homoserine dehydrogenase (HSDH), an important enzyme for many industrial bioprocesses, to demonstrate the feasibility of reengineering enzyme allostery.

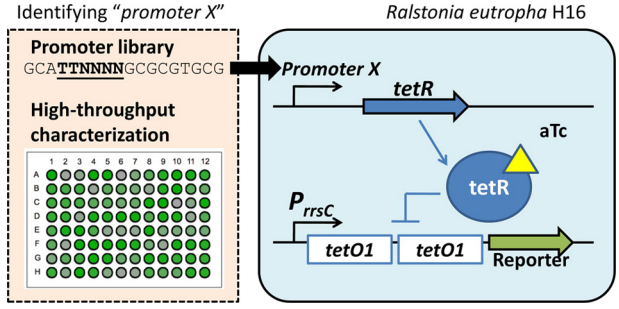
HSDH is naturally, allosterically regulated by threonine and isoleucine. Here, authors re-engineer HSDH, by inserting targeted mutations, to respond to an unnatural inhibitor, L-lysine. This work is of interest in the field of synthetic biology for the reprogramming of cellular metabolism for industrial biotechnological applications. Additionally, the approach described here might also serve as inspiration for the rational modification of other enzymes in order to achieve dynamic control of metabolic fluxes.

DESIGN AND IMPLEMENTATION OF A BIOMOLECULAR CONCENTRATION TRACKER



Implementing reliable feedback and control in engineered circuits remains a challenge in the field of synthetic biology. While positive and negative feedback loops are commonplace in natural biological networks, synthetic circuits depend primarily on library-based screening to achieve optimal expression levels. Here, Hsiao *et al.* (DOI: 10.1021/sb500024b) describe the conceptualization, mathematical modeling, and experimental performance of an *in vivo* protein concentration tracking circuit, in which the concentration of an output protein closely follows that of an inducible reference protein. The authors use protein scaffolds and negative feedback to achieve fast real-time tracking of molecules entirely within the cell.

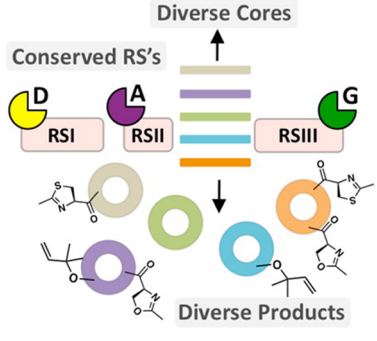
SYNTHETIC ANHYDROTETRACYCLINE-CONTROLLABLE GENE EXPRESSION SYSTEM



Ralstonia eutropha H16, a Gram-negative, facultative lithoautotrophic bacterium, is an organism of biotechnological importance. While recent metabolic engineering work has demonstrated the production of biofuels in *R. eutropha*, more advanced work requires tools such as a controllable gene expression system. Here, Li and Liao (DOI: 10.1021/sb4001189) report the development of an anhydrotetracycline (aTc)-controllable gene expression system in *R. eutropha* H16, which can be gradually regulated with different aTc concentrations with over 10-fold dynamic range.

Using a reporter-activity based promoter library screen, the authors first identified active hybrids between the *tetO* operators and the *R. eutropha* native *rrs*C promoter and then showed that the hybrid promoters are repressable by TetR. To optimize the dynamic range of the system, a high-throughput screening of 300 mutants of *R. eutropha pha*C1 promoter was conducted to identify suitable promoters to tune the *tetR* expression level. This aTc-controllable gene expression system is a useful synthetic biology tool for future scientific research and metabolic engineering in *R. eutropha* H16.

RECOGNITION SEQUENCES AND SUBSTRATE EVOLUTION IN CYANOBACTIN BIOSYNTHESIS



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Ribosomal peptide natural products (RiPPs) comprise one of the largest groups of bioactive natural products. These peptides are compelling because they can be synthesized via a simple ribosomal mechanism but possess key drug properties due to extensive posttranslational processing. The RiPP cyanobactin pathways *pat* and *tru* have been experimentally shown to be extremely tolerant of mutations. In this paper, Sardar *et al.* (DOI: 10.1021/sb500019b) work toward determining the mechanism behind why, enzymes remaining constant, substrates are hypervariable and readily evolvable in these pathways.

By analyzing different enzyme−substrate combinations, as well as engineered substrates, the authors identified short, portable recognition sequences, within the substrate, that are responsible for directing enzymes. Since these features provide the potential for fine control of posttranslational modifications in peptides, this work is valuable to the portion of the synthetic biology field that seeks to control *in vivo* chemical design.

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