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Editorial

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Special Issue on Circuits in Metabolic Engineering

The overall goal of metabolic engineering is to hijack cellular metabolism for the overproduction of useful molecules in engineered host cells, including pharmaceuticals, commodity chemicals, biofuels, and material precursors. For metabolic production to be scalable and economically viable, engineered cells must be robust enough to produce target molecules with high titers, yields, and productivities under a set of fermentation conditions—a historically challenging goal for metabolic engineering. Recent development of synthetic biology has provided a variety of tools to control transcription, translation, and protein activity. These tools have been extensively used in metabolic engineering to regulate expression level and activity of key enzymes involved in metabolic pathways, providing static control of metabolic flux under a predefined condition.

A key component of synthetic biology is gene circuitry, which allows engineered cells to sense extracellular or intracellular signals and perform tasks (such as gene expression) accordingly. Gene circuits that sense metabolite-relevant signals are particularly useful to metabolic engineering because they enable dynamic regulation of metabolic flux. Dynamic regulation allows engineered cells to simultaneously adapt their metabolic flux to different conditions and growth states. Such control optimizes both the amplitude and timing of gene expression and prevents the overaccumulation of metabolic intermediates to unnecessary levels, thereby improving both productivity and strain robustness. This special issue of *ACS Synthetic Biology* focuses on the development of various circuits for use in metabolic engineering, particularly for dynamic regulation.

Enzymes are the key components of metabolic pathways. One of the most fundamental approaches to regulate metabolic flux is to control concentrations or functions of single metabolic enzymes. Traditional approaches in controlling enzyme/protein activities involved the use of constitutive or inducible promoters, which can only be optimized for a single set of fermentation conditions, and cannot accommodate stochastic cell-to-cell variations in protein concentrations. In this issue, Hsiao *et al.* developed a protein-scaffold-based negative feedback circuit to track cellular protein concentrations. Excess amount of the input protein induced colocalization of a two-component system and resulted in the production of an antiscaffold protein that binds and inhibits the input protein. The system allows dynamic tracking of protein concentration in *Escherichia coli*. With additional engineering effort, it is likely that the system may be adopted to control protein concentrations dynamically during cell growth.

Another approach to control metabolic enzymes is to regulate protein activities allosterically. Chen *et al.* demonstrated dynamic control of enzyme activity using a protein engineering approach. Here, they targeted a *Corynebacterium glutamicum* homoserine dehydrogenase (HSDH), a key enzyme that directs metabolic flux to competing pathways of lysine bioproduction. HSDH is naturally inhibited by threonine and isoleucine. Through mutagenesis, an engineered HSDH was created, whose activity was allosterically inhibited by lysine. The engineered HSDH can be used to control lysine production, allowing cells to synthesize other amino acids for growth before lysine production is turned on, and decreasing flux toward competing pathways after turning on lysine production, thereby increasing lysine yield.

Compared to control at protein level, dynamic regulation at the pathway level is more advantageous as it simultaneously optimizes concentrations of mRNA, protein, and metabolite. However, dynamic regulation of metabolic pathways is more difficult than regulating single proteins because of the vast possibilities in regulation topologies and complicated interactions between metabolites and control circuits. Toward solving the first problem, Stevens *et al.* used mathematical modeling to investigate the potential for RNA-based metabolic circuits and applied them to an engineered *p*-aminostyrene biosynthetic pathway in *E. coli*. By formulating 729 unique control topologies and performing a total of 3×10^6 simulations using a broad range of parameter settings, they discovered generalized trends in successful control topologies and found that aptazyme-based dynamic regulation can yield >10-fold improvement over static control. This study has provided guidance that will be very useful for construction of RNA-based dynamic regulation experimentally. Additionally, Rogers *et al.* developed a split-Spinach aptamer system that might be further engineered to create RNA-based metabolic circuits.

My own research group demonstrated the successful design, construction, and characterization of a metabolic feedback circuit that was used to dynamically control metabolic pathways involved with malonyl-CoA. Malonyl-CoA is a key precursor for the biosynthesis of fatty acids, polyketides, flavonoids, and many other industrially important molecules. Malonyl-CoA is synthesized from acetyl-CoA by acetyl-CoA carboxylase (encoded by *acc*), which is the rate limiting step for fatty acid biosynthesis. Overexpression of *acc* improves fatty acid production, but also slows down cell growth. We engineered a metabolic circuit that constantly detects cellular malonyl-CoA concentration and controls *acc* expression according to malonyl-CoA concentration. This negative feedback circuit was able to adjust cellular malonyl-CoA concentrations automatically and dynamically. We demonstrated that the metabolic circuit not only was able to alleviate the toxicity associated with *acc* expression, but also increased fatty acid production.

Because of the presence of stochastic cellular events, such as transcription, metabolite concentrations can vary between cells (*i.e.*, metabolic noise) and propagate through metabolic pathways—a serious but overlooked problem. Negative feedback gene circuits have been shown to decrease noise in gene expression, but whether a metabolic circuit is able to decrease

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metabolic noise propagated through a metabolic pathway is not known. Oyarzún *et al.* performed the first theoretical study and revealed the ability of a metabolic circuit to attenuate noise. They also quantified the impact of design parameters in feedback circuits (repression strength and promoter strength) on the noise size and have proposed design criteria to reduce cell-to-cell variability. This study has provided valuable theoretical basis and design rules for using metabolic circuits to reduce noise in metabolic pathways.

Feedback circuits are also prevalent in nature. Sometimes they have to be rewired to eliminate undesirable single-cell behaviors, such as all-or-none induction of gene expression. This is particularly important when engineering nonmodel organisms, as few reliable control tools are available for these hosts. Afroz *et al.* studied the arabinose utilization pathways with the aim to engineer them as titratable induction systems. Using a combination of modeling and single-cell measurements, they found that there are trade-offs in engineering sugar utilization pathways for titratable control and have suggested a few design rules for manipulating such pathways. On the other hand, Li *et al.* used the TerR-tetO-based circuits and engineered a library of inducible promoters to control gene expression in *Ralstonia eutropha*, a lithoautotrophic bacterium that has been engineered for the production of biofuels from a variety of feedstocks, including sugars, H_2, formic acid, or electricity and CO_2. They demonstrated a significantly decreased basal expression level and a greater than 10-fold dynamic range when titrated with different anhydrotetracycline concentrations.

There is no doubt that synthetic biology will continue to offer more circuits to metabolic engineering, making engineered pathways and cells perform more efficiently and effectively. To achieve this goal, we will need more metabolic sensors with tunable dynamic ranges and sensitivities, more accurate models to guide circuit design, and better understanding of the interplay between circuits and metabolism to simplify circuit construction and fine-tuning. It is hopeful that in the near future, synthetic circuit systems as complex as natural regulatory networks can be engineered and applied to metabolic engineering, facilitating industrial-scale production of many valuable molecules.

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Notes

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