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Editorial

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Metabolic Engineering and Synthetic Biology in Strain Development

Every year, we consume about 27 billion barrels of fossil oil. This enormous amount of oil is used for fueling our cars and airplanes and also for making the various chemicals and materials we use every day. Although other resources, such as shale gas, are becoming available as new energy and chemical sources, these fossil resources will not be available in the future, simply because we consume them at a much higher rate than the rate at which they are formed. Furthermore, environmental problems and climate change issues are urging us to move away from our high dependence on the fossil resources. To reflect the change required, there has recently been much interest in producing chemicals and materials from renewable non-food biomass through biorefineries. For several reasons, microorganisms can be used as factories for the bio-based production of chemicals and materials. However, microorganisms isolated from nature are in most cases not capable of producing our desired bioproducts at high enough efficiencies. This is where metabolic engineering and synthetic biology come into play – to enhance the capability of bioproduct formation at the cellular level as well as the bioprocess level.

Metabolic engineering, defined as the purposeful modification of cellular and metabolic networks to achieve goals such as the enhanced production of desired bioproducts using various tools like recombinant DNA technology, has been around for more than two decades. It has been playing increasingly important roles in designing and developing microbial and mammalian cells that are capable of producing desired bioproducts with high efficiencies. More recently, synthetic biology has emerged with our ability to synthesize DNA at low costs and to modularize and fine-tune gene structure and expression. Both disciplines complement each other in the development of more efficient cell factories for the production of chemicals, fuels, and materials from renewable resources. This special issue puts together contributions by leading scientists and engineers to share the strategies and approaches that can be taken to develop efficient strains.

Gregory Stephanopoulos, one of the pioneers of metabolic engineering, first dives deep into the comparison of metabolic engineering and synthetic biology. Both unique and complementary roles are discussed. The two disciplines are nicely analyzed in the context of chemical engineering unit operations. He suggests that synthetic biology will be much benefited by adopting the chemi-centric, unit operations-based paradigm of metabolic engineering.

Jay Keasling, a leading synthetic biologist and metabolic engineer, has developed a number of groundbreaking microbial systems for the production of several different advanced fuels. In this review paper, an overview of the biosynthetic pathways devised in the strain development of various biofuel-producing microorganisms is presented. Focus is given to strategies that have been employed to identify and overcome pathway bottlenecks and problems of toxicity in addition to creative pathway designs that maximize biofuel production.

Ramon Gonzalez and his colleagues previously reported the engineered reversal of the β-oxidation cycle as a new platform for the production of fuels and chemicals by engineering global regulators and eliminating native fermentative pathways. In their subsequent work, reported in this issue, they address the improvement of this system by using a synthetic biology approach to construct and functionally characterize a reversal of the β-oxidation cycle. The functional units of the pathways are characterized in depth through *in vitro* kinetic studies followed by *in vivo* assembly. Through this approach, the synthesis of a variety of four-carbon carboxylic acids from a one-turn functional reversal of the β-oxidation cycle was realized. It is claimed that the engineered reversal of the β-oxidation cycle is readily transferrable to the host of interest for efficient production of chemicals and fuels.

Light is a well-adapted stimulus to achieve fine control as demonstrated by the light-actuated chemical and biological systems in recent years. Damien Baigl and colleagues report an interesting synthetic biology approach to photocontrol the conversion of β-lactams into β-amino acids by the addition of a cationic photosensitive surfactant to a gene expression medium containing DNA coding for β-lactamase. Conversion of substrate to product was finely modulated by light. Furthermore, spatially resolved activation of substrate conversion was demonstrated by employing independent microfluidic chambers. Triggering chemical reactions through a light stimulus is an interesting strategy for various chemical and biological applications.

My own group reports the design and construction of a 100% rationally engineered *E. coli* strain capable of overproducing one of the branched amino acids, L-isoleucine. By employing systems biology approaches to identify the rate controlling and key regulation steps, an *E. coli* strain was consequently engineered in a stepwise manner, at the systems-level, to achieve enhanced L-isoleucine production. The final engineered *E. coli* strain was able to produce 9.46 g/L of L-isoleucine. This is an example of how to design an optimally performing microbial strain by combining metabolic engineering with synthetic biology and systems biology (thus termed systems metabolic engineering) for the production of a highly regulated product.

Synthetic biology allows for the creation and optimization of many interesting pathways and regulatory circuits for industrial applications. However, not many studies have evaluated how such synthetic circuits actually perform in real industrial production systems. Christopher Voigt, together with industrial partners at DSM, reports interesting work on how synthetically designed circuits perform under industrially relevant conditions. For the successful industrial applications, the genetic circuits designed should be robust enough to cope with varying and complex environments. Using synthetic AND and NOR gates in *E. coli* as examples they report that these two circuits perform differently in different strains and different media. Also, the

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scale-up process showed some challenges in using such designed circuits. These results are of great importance as they highlight challenges that need to be addressed as well as the necessity to develop modified strategies for constructing genetic circuits that function reliably during industrial fermentation.

Increasingly diverse and sophisticated synthetic biology tools are being developed. These new tools can be integrated into metabolic engineering for many benefits such as fine control of metabolic fluxes, robust control of regulatory circuits, generation of more efficient enzyme cascade reactions, creation of metabolic reactions that do not exist in nature, and many others. The current successful examples of biobased production of amino acids, nucleotides, antibiotics, ethanol, lactic acid, succinic acid, 1,3-propanediol, 1,4-butanediol, and polyesters form the first short list of what high performance designer microorganisms can deliver. It is expected that many other chemicals and materials will be produced thorough biorefineries employing "super-microbial factories", which can most effectively be developed by integrating metabolic engineering with synthetic biology.

It is hoped that the readers of this special issue will be able to learn or recap the general and specific strategies employed to develop high performance strains and, more importantly, have a chance to think about what the next strategies for further improvement of strains could be. Before closing, I would like to thank all the contributors of this special issue.

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