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Opinion

# Synthetic Evolution of Metabolic Productivity Using Biosensors

Thomas C. Williams,[1] Isak S. Pretorius,[1] and Ian T. Paulsen[1,*]

**Synthetic biology has progressed to the point where genes that encode whole metabolic pathways and even genomes can be manufactured and brought to life. This impressive ability to synthesise and assemble DNA is not yet matched by an ability to predictively engineer biology. These difficulties exist because biological systems are often overwhelmingly complex, having evolved to facilitate growth and survival rather than specific engineering objectives such as the optimisation of biochemical production. A promising and revolutionary solution to this problem is to harness the process of evolution to create microbial strains with desired properties. The tools of systems biology can then be applied to understand the principles of biological design, bringing synthetic biology closer to becoming a predictive engineering discipline.**

## Design in Synthetic Biology Is Limited by Biological Understanding

Synthetic biologists currently possess an unprecedented capacity to construct large DNA sequences. Products of this construction process range from individual genes and promoters through to entire metabolic pathways and microbial genomes [1,2]. Although it is now possible to assemble synthetic DNA sequences of virtually any size, there is still a limited capacity to design biological systems that deviate significantly from their naturally occurring counterparts. This difficulty exists due to the complexity of biological systems and our incomplete understanding of genotype to phenotype relationships. One promising mechanism to circumvent these limitations in synthetic biology is to use the process of evolution to achieve engineering objectives. Evolutionary trajectories and their genetic basis can then be documented using the various omics tools of systems biology. In theory, this process can be used to learn new principles for the rational design of biological systems. This 'reverse engineering' is beginning to be implemented in microorganisms for the production of valuable biofuels, chemicals, pharmaceuticals, and flavours and fragrances, where the complexity of biological systems is a constant barrier to the success of metabolic engineering efforts.

Current methods for overproducing target biomolecules involve the overexpression of relevant metabolic pathway genes, the elimination of enzymes that compete for carbon, and the balancing of ATP and reducing power (NADH and NADPH) [3]. High-profile successes in the field include the commercial production of the antimalarial compound artemisinin in the yeast *Saccharomyces cerevisiae* [4] and the production of the important industrial polymer 1,3-propanediol in *Escherichia coli* [5–7]. Developing microorganisms into 'cellular factories' that produce a desired, non-native product at commercial **yields** (see Glossary) usually requires many millions of dollars, hundreds of person-years, and highly diverse expertise. This is because progress occurs as part of an iterative and time-consuming design/build/test cycle that is akin to a classical trial and error method. Although there are exceptions to this situation, such as the rational engineering of *E. coli* for the production of 1,4-butanediol [8], our

## Trends

Synthetic biological systems can range in size and complexity from metabolic pathways to entire genomes.

Our capacity to assemble DNA sequences is not matched by an ability to predictively engineer novel biological functions because of the overwhelming complexity of biological systems.

Adaptive laboratory evolution (ALE) allows systems-biology approaches to be used to discover the genetic and physiological basis of evolved phenotypes, thereby informing rational design.

If ALE could be applied to evolve microbes for the production of target metabolites, then many of the bottlenecks that currently limit rational engineering in synthetic biology could be overcome.

Metabolite biosensors connect the intracellular concentration of a target molecule to a survival output. Genetically diverse populations can then be screened for superior producers that have novel genomic architectures.

[1]Department of Chemistry and Biomolecular Sciences, Macquarie University, NSW, Australia

*Correspondence: ian.paulsen@mq.edu.au (I.T. Paulsen).

incomplete understanding of biology still limits the speed at which metabolic engineering can proceed.

## The Evolution of Metabolism

All rational metabolic engineering efforts are fundamentally limited by the fact that a given organism has a genome that has evolved to execute survival and proliferation functions. From the first chemical information systems in the early biosphere to today's modern genomes of extraordinary complexity, life has been governed by the imperatives of replication and survival [9]. The very best metabolic engineering efforts have rendered target chemical production the primary byproduct of normal growth-based metabolism. The ultimate goal of metabolic engineering is to make target compound production the primary function of an organism. Presently this is not possible due a fundamental lack of understanding of evolved biological complexity. However, the use of evolution itself to achieve engineering objectives is a powerful asset that sets synthetic biology aside from other engineering disciplines [10].

The process of evolution can be used not only to produce superior strains but also to inform rational design. By sequencing the genes and genomes of cells isolated from evolving populations it is now possible to pinpoint the time at which advantageous mutations arise (Box 1). By interrogating the nature of evolved mutations using tools such as transcriptomics, proteomics, and metabolomics, genotype–phenotype relationships can now be understood with unprecedented clarity. This approach has been used to investigate the mechanisms of various stress tolerance phenotypes in industrial microorganisms, and is beginning to be implemented for the evolution of metabolically productive genomes.

## Controlling Laboratory Evolution with Biosensors

**Adaptive laboratory evolution** (ALE) is a widely used and highly effective tool in metabolic engineering for creating industrial strains with superior properties (see Figure I in Box 1). If ALE could be used to evolve **metabolic productivity**, then many of the challenges of traditional metabolic engineering could be overcome. Productive strains could simply be evolved, reducing the significant time and cost currently necessary to achieve commercial yields. Most importantly, the evolutionary process could be documented using whole-genome sequencing and the tools of systems biology (Box 1). The interrogation of the genotype–phenotype relationship in evolved strains could then be used to inform rational design with completely novel engineering principles. For example, mutations in genes involved in cellular processes that are currently considered peripheral to metabolic productivity could be hugely important. It is possible that cellular processes/features such as ribosome biogenesis, the cell cycle, cellular morphology, or cell membrane composition greatly affect the metabolic flux towards a particular compound. The process of synthetic evolution would reveal such phenomena.

The most immediate challenge associated with using **biosensors** to evolve productive genomes lies in converting the concentration of a desired compound into an output for cell survival. Much of synthetic biology is concerned with the engineering of tailored responses to biological signals [11,12], and small-molecule biosensors have great potential for achieving these goals [13] (Box 2). The coupling of cell survival to target **metabolite** concentration is beginning to be realised in the form of *in vivo* biosensors (see Figure IA in Box 2), and these biosensors are now being used to select for novel and productive microbial genes and genomes.

## Transcriptional Regulator-Mediated Biosensing

Most biosensors fall under two main categories, allosterically controlled **transcriptional regulators** (TRs), or RNA secondary structures with metabolite specific ligands (see Figure IB,C in Box 2). TRs are an obvious choice for metabolite biosensors because the bacterial domain of life is replete with small-molecule regulated transcriptional repressors and activators [14–16]. The

## Glossary

**Adaptive laboratory evolution (ALE):** a process in which a dividing population of microorganisms evolves tolerance to a selection pressure over time.
**Biosensor:** any molecular device or structure that can sense a molecule of interest and output a detectable signal in response.
**Fluorescence-activated cell sorting (FACS):** individual cells can be separated and cultured from a mixed population based on their fluorescence.
**Fluorescence-activated droplet sorting (FADS):** similar to FACS, except that cells are encapsulated within oil droplets such that components outside of individual cells can be measured.
**Green fluorescent protein (GFP):** used as a selective marker in conjunction with FACS.
**High-throughout screening (HTS):** where desired strain characteristics such as metabolic productivity can be selected from large libraries of genetically diverse cells.
**Metabolic productivity:** the capacity of a particular metabolic network to convert a carbon source into a metabolite of commercial interest.
**Metabolite:** an organic molecule involved in metabolism, many of which can be used in industry as biofuels, pharmaceuticals, or chemicals.
**Ribosome binding site (RBS):** a site in an mRNA molecule that enables translation via association with a ribosome.
**Single-nucleotide polymorphism (SNP):** caused by DNA mutagenesis or replication errors.
**Titre:** the concentration of a particular compound. In this article 'titre' refers to the concentration of an industrially important metabolite in a microbial culture.
**Transcriptional regulator (TR):** typically a protein that binds to DNA to promote the transcription of a downstream open reading frame (ORF).
**Yellow fluorescent protein (YFP):** used in the same way as GFP.
**Yield:** the amount of target metabolite produced per amount of carbon source provided for the microbial culture.