Chapter 1
Introduction

Stephen Van Dien

Abstract The field of metabolic engineering, loosely defined as the manipulation of living organisms to achieve a desired metabolic objective, has grown and advanced significantly over the past 20 years. First applied to improve organisms producing existing biochemicals, it is now a promising approach to develop biocatalysts for the production of nonnatural fuels and chemicals previously accessible only through petrochemical processes. New tools such as gene synthesis, advanced cloning techniques, 'omics' analysis, and mathematical modeling have greatly accelerated the pace of innovation in the field, leading to many success stories and even some commercialization examples. This volume reviews the current state of the art in tools and technologies for metabolic engineering.

In October 1996, a group of 100 or so microbiologists, molecular biologists, and chemical engineers gathered at a conference resort outside of Boston to discuss the emerging field of Metabolic Engineering. Recombinant DNA technology was well established at this time, particularly for the expression of industrial and pharmaceutical proteins, and the idea of manipulating an organism's metabolic network was starting to take shape. As defined in a landmark paper by the late Professor James 'Jay' Bailey several years earlier (Bailey 1991), metabolic engineering was distinguished from genetic engineering by the need to express multiple genes that form a pathway, rather than a single protein. The intended product is not the enzymes themselves, but rather the result of their function; i.e., metabolism. With the belief that all metabolic pathways had been elucidated already, metabolism research had fallen out of fashion in the 1970s and 1980s, while structural biology and genetic regulation became the hot topics. Even the undergraduate biochemistry course I had taken glossed over the chapters on amino acid and nucleotide biosynthetic pathways, to spend more time on molecular biology. Technology for manufacture of biochemicals, from ethanol to amino acids to antibiotics, was largely mature. Production organisms were obtained using classical mutagenesis and

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screening, starting from a native producer, and most of the development in biochemical engineering was on cost optimization of the process, not the organism. Many of the early pioneers in metabolic engineering research were trained in fermentation, process design, and separations. When directed genetic manipulations became commonplace in the late 1980s for the development of modified enzymes via site directed mutagenesis, it wasn't long before the implications to modifying entire pathways were realized. There was a resurgence of interest in metabolism, and a new field was born.

I had the privilege of attending this conference in Massachusetts, now known as Metabolic Engineering I, as a graduate student; I was nearly star-struck by the opportunity to not only see lectures by, but also to share casual dinner conversation with top researchers in my field. Before the meeting, I had a vague idea that my thesis project to selectively control the expression of two genes, encoding the synthesis and degradation of a simple polymer, was considered metabolic engineering. I knew very little about the implications pathway manipulation could have for biochemical production. Being from Texas, and with an undergraduate degree in Chemical Engineering, my naïve understanding was that all chemicals came from oil and gas. By the end of the meeting, I had found a scientific home. Not only was I fascinated with the unlimited potential of microbial metabolism, but I also loved the intersection of mathematical and engineering concepts with molecular biology. A cartoon shown in one of the presentations joked that the difference between genetic engineering and metabolic engineering is "lots and lots of math".

Although the science has advanced significantly in the last 20 years, some of the themes of that first meeting are still relevant today. A major area of focus was the development of computational tools for both measurement and design. Just as mechanical or chemical engineers use theory and mathematical models to design products and processes, metabolic engineers sought to follow the same paradigm. Flux balance analysis (Schilling et al. 1999; Schilling et al. 2000) and metabolic control analysis (Fell and Sauro 1985) had engineering parallels in process optimization and process control, respectively. The underlying theory, based on the stoichiometry of metabolic networks, had been developed more than 20 years earlier by Kacser and Burns (1973), and was now finding application not only in understanding metabolism, but also in manipulating metabolism. Still relevant today, stoichiometric modeling has been used to guide development of novel organisms for commercial bioprocessing (Yim et al. 2011). Introducing an exogenous pathway, or enhancing expression of a native one, is not sufficient for achieving commercial metrics. Metabolic engineering involves re-routing central metabolism to supply precursor molecules, balancing reducing equivalents, and proper tuning of the pathway expression. Once basic strain designs are generated with modeling, genetic tools are needed to implement designs and experimental techniques required to measure the output. Artificial promoters, gene integration techniques, transcript profiling, and the elucidation of complex regulatory circuits were all discussed at the 1996 meeting. Applications of metabolic engineering covered a broad spectrum. Key products of interest were pharmaceuticals, amino acids, modified fatty acids, the natural biodegradable polymer polyhydroxybutyrate,

and solvents. Perhaps envisioning the cellulosic biofuels boom that would come a decade later, work was presented on C5/C6 sugar co-utilization. Notably absent were nonnatural chemicals such as isobutanol or 1,4-butanediol. Other applications of metabolic engineering principles included the study of human tissues, cell culture, plants, and xenobiotic degradation. Nowadays, with a few exceptions the term metabolic engineering is usually understood to mean applications in microbial bioproduction.

With the turn of the century came the era of functional genomics, where nearly complete genome sequences and microarrays of model organisms were commonplace. Gene sequencing (Sanger) and oligonucleotide synthesis were available as services, many molecular biology protocols could be purchased as kits, and PCR machine throughput increased; consequently, the pace of research accelerated. Success stories like 1,3-propanediol (Nakamura and Whited 2003) were starting to emerge, catching the attention of researchers and chemical companies alike. The excitement and opportunity around metabolic engineering was captured by Professor Bailey in a video presentation of his acceptance speech for the First Merck Award in Metabolic Engineering at the Metabolic Engineering III conference in October, 2000, a few months before his death (Bailey 2001). His vision was captured in a song:

The (Metabolic Engineering) Times, They Are A 'Changin'
(Bob Dylan/Jay Bailey)

* Reproduced from Stephanopoulos (2001)

Come gather Metabolic Engineers 'cross the land
At MEIII we'll take command
Of cells that are too slow to produce or grow.
If it's higher fluxes you're needin'
Then we'll shift the controls, and block bad outflows.
For the times, they are a changin'.

Do you need a new molecule or neutraceutical
The Metabolic Engineer has the answers for you.
We'll import new pathways, and shuffle them too.
Is your lead compound library fadin'?
We'll give new adducts to your old natural products
For the times, they are a changin'.

Rational or random, which way is best?
Solving the problem passes the test.
Complex responses confuse the quest.
More genetic and array technologies
Will give us insights to networks' delights.
For the times, they are a changin'.

Genomes are in hand, the sequences there,
An amazing resource that we all share.
Genes and controllers, bioinformatics tells us where.
But how is all of this workin'?

Let's decipher a yeast, understand that at least.
For the times, they are a changin'.

How is phenotype controlled by the genes?
Nobody knows, least of all the machines.
Medicine will thrive if we can discover the means,
To merge our knowledge and information
And find genes' intent and control by environment.
For the times, they are a changin'.

Metabolic Engineers have all the tools
Biology, computing, and engineering rules,
Knowledge, experience, perspective on detail.
Let's help Metabolic Genomics to set sail.
Opportunity's here . . . but now it's time for a beer!
For the times, they are a changin'.

The times continued to change. The next decade brought NextGen sequencing, gene synthesis, and the rise of synthetic biology. The number of genomes sequenced, and consequently the unique gene sequences in GenBank and other databases, grew exponentially. This provided more choices for assembling genes into pathways, and these genes could now be custom ordered with codon-optimized sequences to improve expression in the host production organism. Inexpensive whole-genome sequencing enabled the deconvolution of mutations from adaptive evolution experiments, as well as RNAseq to routinely monitor transcriptome profiles even more efficiently than microarrays. Other 'omics' techniques—proteomics, metabolomics, and ^{13}C-flux analysis—also found applications for diagnosing bottlenecks in production strains (Van Dien 2013). New success stories for diverse products including artemisinin (Westfall et al. 2012), 1,4-butanediol (Yim et al. 2011), lactic acid, and isobutanol (Atsumi et al. 2009) emerged.

Changes in our approach to metabolic engineering are still on the horizon, driven both by technological advances that improve the way we do science and market forces that influence the product choices. This volume includes workflows and methodologies based on state-of-the-art technology in 2016. There will certainly be cases where the methodologies described are no longer practiced several years from now; although the specific methods may change, the overall scientific approaches to engineering organisms will continue to be relevant. Specifically, the articles that follow describe approaches to pathway development and tuning, host strain optimization, evolution to overcome stresses, and fermentation process scale-up. Theory and modeling are not covered here; the reader is referred to excellent textbooks on the subject (Stephanopoulos et al. 1998; Patnaik 2013) and the references therein. By application of the methods described in the following sections, we hope to inspire a new generation of metabolic engineering success stories.

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