

Challenges and opportunities in synthetic biology for chemical engineers



Yunzi Luo^a, Jung-Kul Lee^c, Huimin Zhao^{a,b,*}

^a Department of Chemical and Biomolecular Engineering, University of Illinois at Urbana-Champaign, Urbana, IL 61801, United States

^b Institute for Genomic Biology, Departments of Chemistry, Biochemistry, and Bioengineering, University of Illinois at Urbana-Champaign, Urbana, IL 61801, United States

^c Department of Chemical Engineering, Konkuk University, Seoul 143-701, South Korea

HIGHLIGHTS

- ▶ Main challenges and opportunities for chemical engineers in synthetic biology are discussed.
- ▶ Standardization of biological parts represents a key challenge in synthetic biology.
- ▶ Chemical engineers play a leading role in engineering cellular factories.

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ABSTRACT

Synthetic biology provides numerous great opportunities for chemical engineers in the development of new processes for large-scale production of biofuels, value-added chemicals, and protein therapeutics. However, challenges across all scales abound. In particular, the modularization and standardization of the components in a biological system, so-called biological parts, remain the biggest obstacle in synthetic biology. In this perspective, we will discuss the main challenges and opportunities in the rapidly growing synthetic biology field and the important roles that chemical engineers can play in its advancement.

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1. Introduction

Synthetic biology focuses on the design and construction of biological systems that draws on principles elucidated by biologists, chemists, physicists, and engineers. The term synthetic biology is almost as old as the term genetic engineering. However, synthetic biology has recently become a field of its own, mostly driven by the advances in DNA synthesis and sequencing and systems biology (Liang et al., 2011). Synthetic biology has broad applications in agriculture, medical, chemical, and food industries. Examples of landmark accomplishments include microbial production of artemisinic acid, a key precursor of the commonly used antimalarial drug artemisinin (Martin et al., 2003; Ro et al., 2006), commercial

manufacture of bio-derived 1,3-propanediol, an industrial chemical with a variety of applications in solvents, adhesives, resins, detergents, and cosmetics (Nakamura and Whited, 2003; Tang and Zhao, 2009) and the reconstruction of a complete microbial genome by the J. Craig Venter Institute (Gibson et al., 2010).

The key challenges in synthetic biology exist on two main levels. One is the modularization and standardization of biological parts, while the other is the integration of these biological parts into devices with desired functions. Modularization and standardization of biological parts are analogous to modularization and standardization of electronic parts such as inverters, switches, counters, and amplifiers. By doing so, any part can be easily combined with others and reused in genetic devices (Bio FAB Group et al., 2006). Many experimental and computational tools have been developed to address these challenges, creating numerous scientific and technological opportunities. In this perspective, we will briefly highlight these main challenges and opportunities in the rapidly growing synthetic biology field for chemical engineers.

* Corresponding author at: University of Illinois at Urbana-Champaign, Department of Chemical and Biomolecular Engineering, 600 S. Mathews Avenue, Urbana, IL 61801, United States. Tel.: +1 217 333 2631; fax: +1 217 333 5052.

E-mail address: zhao5@illinois.edu (H. Zhao).

2. Challenges in synthetic biology

Luis Serrano, a systems biologist at the Centre for Genomics Regulation in Barcelona, Spain, recently said: “We are still like the Wright Brothers, putting pieces of wood and paper together” (Kwok, 2010). This statement aptly describes the current state of the synthetic biology field. Challenges are manifested at every step in the process of improving an existing biological functionality or creating a new one, ranging from the standardization of the “wood and paper” to the integration of these pieces at the system level.

2.1. Modularization and standardization of biological parts

By snapping together various pieces of different colors, shapes and sizes from a Lego[®] box, a multitude of structures with different functions such as a boat, a car and a building can be readily built. In the world of synthetic biology, biological parts such as genes, promoters and terminators are treated as building blocks in an analogous manner. Improved and novel structures and functions of cells are created by a growing community of scientists who develop and use these building blocks. However, many of the parts are still undefined and incompatible; the circuitry is unpredictable; the complexity is unwieldy; and variability crashes the system sometimes (Kwok, 2010).

All biological parts are encoded by the 4-letter DNA code: ATGC, which can be anything from a gene sequence encoding a specific protein to a promoter sequence regulating gene expression. In 2003, the Registry of Standard Biological Parts was established at the Massachusetts Institute of Technology. Relatedly, in 2004, the BioBricks Foundation (BBF) was established and the International Genetically Engineered Machine (iGEM) competition was started. Numerous new biological parts have been generated through these coordinated efforts for synthetic biology applications. However, most of them are not well characterized. This is mainly because there are many different interactions

under various cellular backgrounds between most of the biological parts, and many of these interactions are not understood. For example, transferring a particular gene with known function in a heterologous host to confer new abilities is often not guaranteed. Similarly, even if particular enzymes are known to catalyze certain reactions, the naturally occurring enzymes are often incompatible or suboptimal for use in a non-native environment. They may lack desired substrate specificity or may be insoluble in desired reaction conditions (Martin et al., 2009). Thus, it is critical to improve the quality of the standard parts and increase the number of reusable parts (Collins, 2012; Grunberg and Serrano, 2010; Martin et al., 2009; Pleiss, 2011).

In addition to small biological parts mentioned above as genes or promoters, some larger parts are also included in the synthetic biology toolkit such as pathways or even whole cells. Not surprisingly, the standardization problem dwells in those parts too. For pathways, the main aim is to create novel or improved biological routes for the production of natural or unnatural compounds. Therefore, one key challenge is the construction and optimization of highly efficient pathways. Because a pathway contains so many genes, promoters, and terminators as well as other regulatory elements that are not well characterized, it becomes very challenging to reliably construct a target pathway with desired features. On the other hand, as a standardized part, a target pathway should be transferable between different hosts. However, most of the known pathways cannot meet this requirement because of the difference in the genetic backgrounds of hosts.

2.2. Integration of biological parts at the system level

Similar to the standardization challenge, integration of various biological parts into a functional biological system has also met many challenges. One challenge is related to the compatibility issue because different biological parts have evolved to fit different contexts. Moreover, the same biological parts may

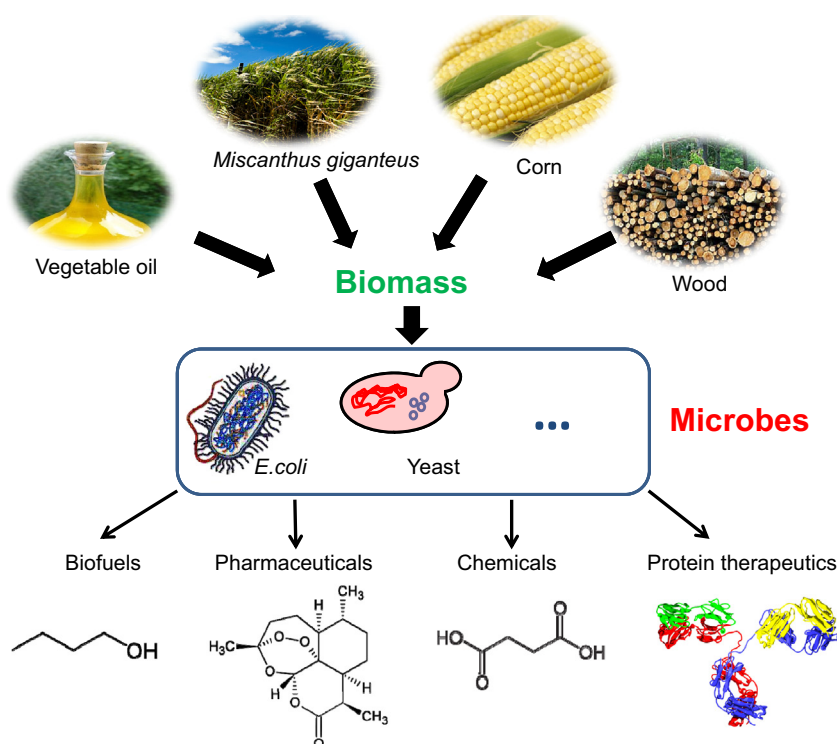


Fig. 1. Conversion of plant biomass to value-added products using recombinant microbes engineered by synthetic biology tools.

behave differently in different cellular backgrounds. Therefore, reprogramming cells using synthetic biology requires the interrogation and understanding of the living system's organizational principles (Bashor et al., 2010).

Based on the available biological parts, scientists seek to build microbial cells to convert cheap, renewable resources such as plant biomass to energy-rich molecules or value-added compounds. However, to engineer the chemistry inside a microbial cell is not a straightforward task. It requires well-characterized gene expression systems and hosts for chemical synthesis and a computer-aided design and analysis system (Keasling, 2008). Additional considerations need to be taken, such as the required chemical reaction conditions, co-factor balance, precursor availability, and control of the timing of the expression of different genes.

3. Opportunities for chemical engineers

Although there are many challenges, numerous scientific and technological opportunities exist for synthetic biologists. For chemical engineers, many of these opportunities are related to the engineering of cellular factories to produce value-added products (Du et al., 2011; Sethuraman and Stadheim, 2006) (Fig. 1), which is also considered as the main focus of metabolic engineering. In most cases, both synthetic biology and metabolic engineering are required for the design and construction of cellular factories used for industrial applications. These two fields are closely related yet distinct from each other. The main differences lie in their scope, tools and applications. Metabolic engineering mainly focuses on a single cell with a main goal of maximizing the formation of a specific product using recombinant DNA technologies. In comparison, synthetic biology focuses on a biological system (living and non-living) which could be a single protein or protein complex, a pathway, a biological machinery, a genome, a cell, a cell consortia, a tissue or organ, and an ecosystem. One of the key enabling tools in synthetic biology is DNA synthesis. Synthetic biology aims to not only engineer microorganisms for production of fuels and chemicals, but also understand the origin of life.

Similar to constructing chemical plants, engineering cellular factories requires system-level and quantitative considerations of issues of design, control, and optimization, and involves the direct application of core principles of kinetics, transport, and thermodynamics that are the foundations of chemical engineering. As such, chemical engineers are uniquely suited to pursuing opportunities in this area. Below we will highlight a few successful examples related to microbial production of biofuels, value-added chemicals, and protein therapeutics, which should provide a glimpse of the numerous opportunities that chemical engineers can take.

3.1. Biofuels

Increasing fuel consumption worldwide and concerns on energy security, sustainability, and climate change are the main drivers for the development of biofuels. Synthetic biology can be used to engineer recombinant microorganisms capable of efficiently converting renewable plant biomass to biofuels such as long-chain alcohols (Atsumi et al., 2008), alkanes (Schirmer et al., 2010), and biodiesel and jet fuels (Steen et al., 2010b).

As mentioned above, the biological parts have not been fully defined yet, which makes pathway engineering and optimization challenging. Thus, multiple pathways and hosts are usually tested to achieve desired functions, such as in the case of *n*-butanol production (Atsumi et al., 2008; Inui et al., 2008; Nielsen et al., 2009). *n*-Butanol, usually produced by *Clostridium* species, is a better fuel alternative than ethanol because of its higher energy content, higher octane number, and lower water solubility.

However, it is challenging to improve the titer and yield of *n*-butanol in the native producers (Yan and Liao, 2009). To address this issue, different heterologous hosts such as *Escherichia coli* and *Saccharomyces cerevisiae* were engineered to produce *n*-butanol (Atsumi et al., 2008, 2010; Steen et al., 2008). The engineered *E. coli* strain harboring the *n*-butanol biosynthetic pathway from Clostridia can produce 552 mg/L of *n*-butanol (Atsumi et al., 2008), whereas the engineered *S. cerevisiae* strain harboring a similar *n*-butanol biosynthetic pathway can only produce 2.5 mg/L of *n*-butanol (Steen et al., 2008). Most recently, through extensive metabolic engineering, the titer of *n*-butanol production in *E. coli* was increased to 30 g/L and the yield was 0.287 g/g glucose (Shen et al., 2011), which exceeds the *n*-butanol production in the native host. Other than *n*-butanol, Liao and coworkers investigated the production of long-chain alcohols through existing non-fermentative keto acid pathways as well. Different 2-keto-acid decarboxylases and 2-keto acids from various amino acid synthesis pathways have been chosen to produce long-chain alcohols including isobutanol, 1-butanol, 2-methyl-1-butanol, and 2-phenylethanol (Atsumi et al., 2008, 2010; Connor and Liao, 2008).

In addition to long-chain alcohols, fatty acid-derived fuel alternatives are also potential fuel alternatives. *E. coli* can produce fatty acid metabolites at the commercial productivity of 0.2 g/L/h per gram of cell mass and achieve product-dependent mass yields of 30–35% (Rude and Schirmer, 2009). Recently, Keasling and coworkers engineered recombinant *E. coli* strains to overproduce free fatty acids through cytosolic expression of a native *E. coli* thioesterase and deletion of fatty acid degradation genes (Steen et al., 2010a). After the introduction of ethanol production genes from *Zymomonas mobilis* and overexpression of endogenous wax-ester synthase, direct production of fatty acid ethyl esters (FAEEs) was achieved at 674 mg/L, which was 9.4% of the theoretical yield.

3.2. Value-added chemicals

In addition to biofuels, a wide variety of value-added compounds can also be produced by recombinant microorganisms engineered by synthetic biology and metabolic engineering strategies. For example, the Keasling group successfully engineered a cellular factory based on either *E. coli* or *S. cerevisiae* to cost-effectively produce artemisinin acid, a key precursor of the commonly used antimalarial drug artemisinin (Martin et al., 2003; Ro et al., 2006). Of note, the yeast based process is now being scaled up for commercial production of artemisinin by Sanofi-Aventis (Westfall et al., 2012). In a related example, the Stephanopoulos group engineered a recombinant *E. coli* strain capable of producing taxadiene, the precursor of the widely used anticancer drug taxol (Ajikumar et al., 2010; Leonard et al., 2010). Another prominent example is the production of the platform chemical succinic acid using recombinant *E. coli* (Lee et al., 2006; Sanchez et al., 2005a, b) or recombinant *S. cerevisiae* (Raab et al., 2010). The engineered *E. coli* strains can produce succinic acid from glucose with up to 99.2 g/L (Yu et al., 2011), whereas the engineered *S. cerevisiae* strain can only produce 3.6 g/L succinic acid. In a related study, a recombinant *E. coli* strain was engineered to produce another platform chemical xylitol from a mixture of hemicellulosic sugars (Nair and Zhao, 2010).

Most importantly, two industrial chemicals including 1,3-propanediol (1,3-PD) and lactic acid have been produced on a commercial scale using engineered recombinant organisms. 1,3-PD is a platform chemical with applications in the production of plastics, cosmetics, lubricants, and drugs. Commercial interest in 1,3-PD has grown significantly after Shell (Netherlands) and DuPont (US) commercialized a new 1,3-PD based polyester poly(propylene terephthalate) with properties appropriate for fiber

and textile applications (Zeng and Biebl, 2002). Competition from Shell led DuPont to develop an economically feasible and sustainable 1,3-PD production process. Through the collaboration between DuPont and Genencor International (US), an engineered *E. coli* K12 strain was engineered to convert D-glucose to 1,3-PD directly at a titer of 135 g/L and a weight yield of 51% in D-glucose fed-batch 10 L fermentation (Diaz-Torres et al., 2000; Laffend et al., 1997; Nakamura and Whited, 2003). Later, production of 1,3-PD was achieved in *E. coli* using glycerol as a substrate with an overall yield and productivity of 104.4 g/L and 2.61 g/L/h, with the conversion rate of glycerol 90.2% (g/g glycerol) (Tang et al., 2009).

Lactic acid (also known as 2-hydroxypropanoic acid) is an important industrial chemical with more than 100,000 metric tons produced annually (Xiu and Zeng, 2008). Lactic acid has been used in food, cosmetics, tanning industry, and as an intermediate in pharmaceutical processes. Most importantly, it has been used to produce polylactic acid for biodegradable plastics and environmentally friendly solvent ethyl acetate. Traditionally, lactic acid was produced by *Lactobacillus* strains. Recently, recombinant *E. coli* strains were engineered to ferment glucose or sucrose for the production of lactic acid by deleting genes in the competing pathways, which resulted in over 90 g/L D-lactate with a productivity of 6.75 g/L/h (Zhou et al., 2005). Further metabolic engineering and evolution led to the pure L- and D-lactate production with a yield > 95% and a titer of 100 g/L (Grabar et al., 2006).

3.3. Protein therapeutics

During the last three decades, protein therapeutics such as hormones, vaccines, antibodies, blood factors, and therapeutic enzymes represent one quarter of the newly approved drugs, among which glycoproteins account for approximately 70% (Sethuraman and Stadheim, 2006). Therapeutic proteins were often traditionally isolated from mammalian sources. With the development of genetic engineering tools, the production of therapeutic proteins has shifted into recombinant systems such as mammalian cell lines, bacteria, yeasts, and insect cells. These systems vary greatly with their ability to incorporate different post-translational modifications on the native human proteins. For example, bacteria lack the N-linked glycosylation machinery, which makes them not suitable for glycoprotein production. Chinese hamster ovary (CHO) cells are the most widely used cells for large-scale therapeutic glycoprotein production. However, they have several disadvantages such as slow cell growth rate and limited control over glycosylation (Yuen et al., 2003). Therefore, over the past decade, other hosts such as yeasts, plant cells, and insect cells have also been developed for therapeutic glycoprotein production. For example, a recombinant yeast strain was engineered to produce therapeutic proteins harboring human complex N-glycosylation (Hamilton et al., 2003). The IgG1 antibody produced in yeast showed > 100-fold enhanced receptor binding affinity compared with that produced in CHO cells (Li et al., 2006).

4. Conclusions

Life is programmed in DNA, and now we understand that the coding of ATGC can not only be read, but also be written by synthetic biology. Although challenges abound across all scales from the modularization and standardization of the biological parts to the integration of all parts together at different levels, there are a growing number of opportunities for chemical engineers. Particularly, the potential of synthetic biology for industrial applications, i.e. the design and construction of cellular factories to convert renewable feedstocks to value-added products, is poised to reach fruition. Chemical engineers can not only design and construct new cellular factories but also develop new

technologies for product separation and purification. The synergism between synthetic biology and chemical engineering has been clearly demonstrated in the development of commercial production processes for 1,3-PD (Xiu and Zeng, 2008), lactic acid (Joglekar et al., 2006), and artemisinic acid (Westfall et al., 2012).

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