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Synthetic Biology and its Applications in Medicine

Introduction

The development of novel technologies in the late 19th and early 20th century lead to the creation of major new industries such as the petrochemical, automotive, aviation, and electronic. These industries have improved the lives of billions of people around the globe[1] and propelled civilization forward. During the second half of the 20th century the digital revolution changed the world yet again, with the rise of personal computers and the internet. According to the UK Royal Academy of Engineering, we are on the cusp of another revolution—this one based on synthetic biology[1]. The applications of synthetic biology are broad, ranging from renewable energy production to agriculture. One exciting application that will have profound implications on human health is medicine. This paper will discuss the advent of synthetic biology and its medical applications.

The Science of Synthetic Biology

The first advancements that lead to the field of synthetic biology can be traced to the development of genetic engineering in the early 1970s. The first recombinant DNA molecule was created by Dr. Paul Berg at Stanford University in 1972 by inserting DNA from the bacterial lambda virus into that of the monkey virus SV40[2]. The following year, the first transgenic organism was created by Herbot Boyer and Stanley Cohen by inserting antibiotic resistance genes into E. coli[3]. Rudolf Jaenisch then created the first transgenic mammal by introducing foreign DNA into the embryos of mice[4]. In 1977 scientists at Genentech produced somatostatin in E. coli, and then human insulin in 1978 which had an extraordinary impact on treatment for diabetes[5]. In the early 1980s the ability to amplify DNA was made possible through the technique of polymerase chain reaction (PCR), and soon after automated DNA sequencing was

developed that allowed for large scale genome sequencing efforts such as the Human Genome Project. As our knowledge and capabilities in biology advance, we are gaining the ability to create rather than just discover.

A. Definition

While there are large parallels between genetic engineering and synthetic biology, there is a distinction. Traditional genetic engineering has largely involved the transfer of individual genes from one species to another. Synthetic biology aims to impose a sense of order to the process, and creates complete and novel biosynthesis pathways by composing a symphony of molecular components such as DNA, RNA, proteins, and cells into circuits and networks[6]. This field aims to apply engineering principles, such as standardization, to biology by creating and using libraries of predictable and reliable biological parts, or "BioBricks" (Figure 1), that can be mixed and matched to produce microbes with new metabolic pathways with applications ranging from producing pharmaceuticals, to detecting or breaking down toxic elements, to generating biofuels[6]. As a whole, synthetic biology is broadly defined as the design and construction of new biological parts, devices and systems for useful purposes.

Symbol	BioBrick parts
	Promoter
	Coding sequence
	RBS
	DNA
	Inverter
	Plasmid backbone
	Terminator

Figure 1: Illustration of BioBricks http://en.wikipedia.org/wiki/Synthetic_biology

B. Top-Down and Bottom-Up Approaches

There are two approaches to synthetic biology: top-down and bottom-up[7]. The top-down approach begins with existing organisms or gene sequences, and modifies them by adding particular parts, or removing unnecessary parts to create new or amplified characteristics and functions. This approach borrows properties from one or more living systems to design novel biological machines. Top-down considerations can include selecting the host organism and assessing potential interactions of the synthetic network with existing cellular networks.

The bottom-up approach is comparatively more challenging. With this approach, scientists begin from the basic building blocks of non-living biological parts to build living systems. The Registry of Standard Biological Parts is a result of this approach, which maintains an open catalog of "BioBricks" housed at MIT that can be used as building blocks of biological devices[8]. The bottom-up considerations can include selecting genes and promoters, and using regulatory elements.

Currently, the design of biological systems through synthetic biology incorporates both top-down and bottom-up considerations due to our incomplete knowledge of biology (Figure 2). For instance, a design process would take the following form: a suitable synthetic biological system is first designed using known properties of biological parts through the bottom-up approach. Next, this synthetic system is constructed and inserted into a larger biological context via the top-down approach. The process is an iterative one and is modified until the desired function is achieved[9]. As our knowledge of biology expands, we will be able to design systems with more certainty and predictability.

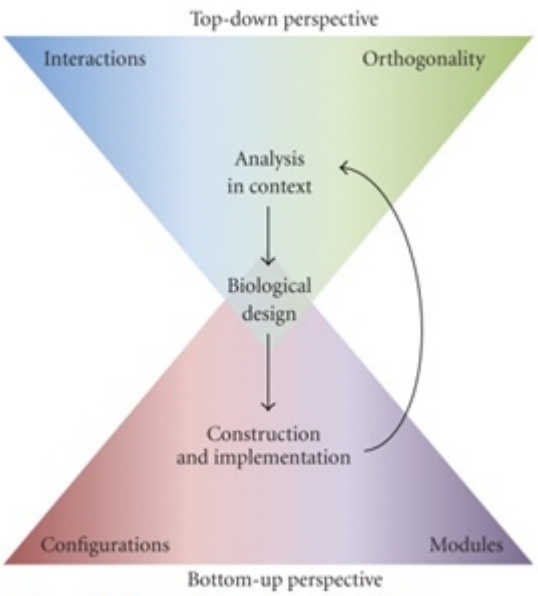

Figure 2: Brian R. Fritz et al, 2010

Synthetic Biology and Medicine

Drug Development

While the field of synthetic biology is still in its infancy, it has already demonstrated great potential in biomedical applications. In drug development, synthetic biology provides many advantages over conventional techniques. For instance, synthetic biology allows the design of cells to screen drug molecules, greatly reducing drug discovery time[6]. Further, metabolic pathways can be more precisely designed and manipulated in synthetic organisms, and cells can

be redesigned to produce desirable molecules with high efficacy and low toxicity. This will not only speed up the drug discovery and development process, but greatly reduce the cost of therapeutic proteins and drugs. Lastly, synthetic biology paves the way for the production of synthetic proteins and molecules for therapeutic purposes.

A. Artemisinin

One specific example is the production of the antimalarial drug artemisinin. This drug is naturally found in the Chinese Sweet Wormwood plant *Artemisia annua* and is very effective in treating malaria. Artemisinin activity involves reductive cleavage of endoperoxide bridges by ferrous iron of digested hemoglobin, producing lethal reactive intermediates in the malarial parasite[10]. The World Health Organization recommends artemisinin combination therapies (ACTs) for treatment against malaria. However, a major problem is the limited availability of artemisinin and the high costs associated with the extraction process from Wormwood plant leaves that make it inaccessible to developing countries[11]. This extraction process is difficult and expensive and increases the cost of the drug.

Luckily, in 2006 a group of researchers at Berkeley lead by Jay Keasling engineered the yeast strain *Saccharomyces cerevisiae* to produce arteminisinic acid, a precursor of artemisinin that tremendously reduced the cost of the anti-malarial drug[12]. They did this through synthetic biology principles by incorporating amorphadiene synthase and cytochrome P450 monoxygenase from the Wormwood plant into yeast, which was also modified to produce large quantities of amorphadiene precursor farnesyl pyrophosphate[9]. Now it is possible to synthesize artemisinin directly, rather than just the precursor, by assembling five plant and yeast genes in a single synthetic expression cluster in a tobacco plant system[13]. Biosynthesis of artemisinin through synthetic biology led to the commercial production of the drug at low cost and large scale via the company Amyris, making it accessible to developing countries (Figure 3).

B. **Taxol**

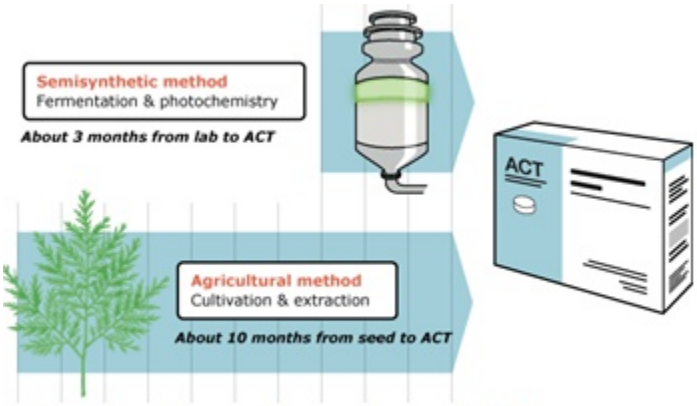

Figure 3: amyris.com/innovation/artemisinin

By the same metabolic engineering approach, researchers were able to produce the anti-cancer agent taxol. The many uses of taxol include therapy for a variety of cancers such as ovarian, breast, bladder, prostate, cervical, and lung cancers. Just like artemisinin, taxol was originally isolated from a plant, the Pacific yew tree, *Taxus brevifolia*. Due to the inefficient extraction processes and low concentration of taxol in Pacific yew trees, the drug's availability was also limited. Moreover, chemical synthesis of taxol is not commercially viable due to low yield and high cost[9]. Fortunately, access to the drug is being enabled through metabolic engineering strategies. Three processes are involved in the biosynthesis of taxol: the formation of a taxane core, additions and modifications of functional groups on the core, and side chain formation. Researchers were able to produce a high titer of taxadiene, the first committed intermediate for the biosynthesis of taxol, by mimicking these processes in E. coli[14].

Vaccine Development

Another area in medicine where synthetic biology can be applied is vaccine development. Traditional development of new vaccines has several drawbacks. The majority of vaccines consist of attenuated or inactivated pathogens. While efficient, these systems have great variation from batch-to-batch and can only be used for pathogens that are readily cultivated in the lab and at scale[15]. Furthermore, there is no control over which antigen will evoke an immune reaction. Mastrobattista et al. used cell-free synthetic biological techniques to design genetically programmable vaccines. In this approach, synthetic vesicles consisting of a lipid bilayer are used as artificial microbes to produce and deliver DNA-encoded antigens (Figure 4). These so-called DNA antigen-expressing immunostimulatory liposomes (AnExILs) have numerous advantages

over conventional vaccines. For example, the specificity of the vaccine can be easily altered by changing the DNA templates used without needing to change the whole vaccine formulation. Furthermore, there is no limit to the number of genes that can be expressed in AnExILs, and AnExILs mimic characteristics of viruses and bacteria without the risks of using attenuated pathogens for vaccinations. In their study, the researchers showed that AnExILs expressing B-galactosidase produced functional antigen proteins in vitro and generated a higher humoral immune response compared to controls after vaccinations in mice. This method presents a promising new platform for DNA-based vaccines that combines antigen production, adjuvant properties, and delivery in one system with many advantages over current vaccine formulations.

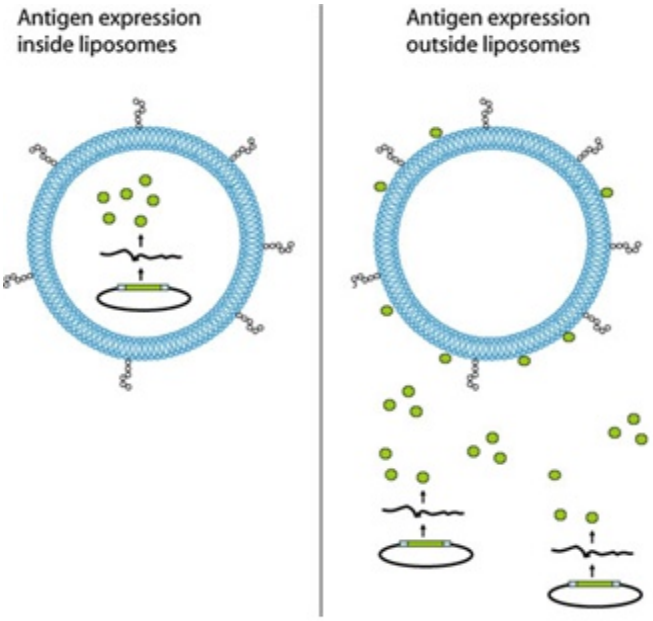

Figure 4: Amidi, Maryam et al, 2010

Microbiome Engineering

The microbiome is an important regulator of human physiology, and consists of over 1000 species that outnumber human cells by factors of ten[16]. These species are great targets for deploying synthetic gene circuits to fight disease and deliver therapeutic molecules directly to the body. One great example is the research done by Duan et al, who used E. coli to prevent cholera infections by engineering a synthetic interaction between gut microbes (Figure 5)[17]. During an infection, *Vibrio cholerae* secretes virulence factors like cholera toxin only at low population densities. Through quorum sensing, *V. cholerae* is able to measure its population density by detecting levels of cholera autoinducer 1 (CAI-1) and autoinducer 2 (AI-2). When both autoinducer levels are high, *V. cholerae* stops expressing virulence factors. By engineering the E. coli strain Nissle 1997, which natively expresses AI-2, to also secrete CAI-1 the researchers were able to prevent *V. cholerae* infection

in infant mice that had ingested the engineered *E. coli.* and increased their survival rate by 77% while reducing cholera toxin intestinal binding by 80%.

In another study by the same researchers, *E. coli* was also engineered to stimulate intestinal epithelial cells to secrete insulin in response to glucagon-like peptide 1 (GLP-1) and PDX-1[18].

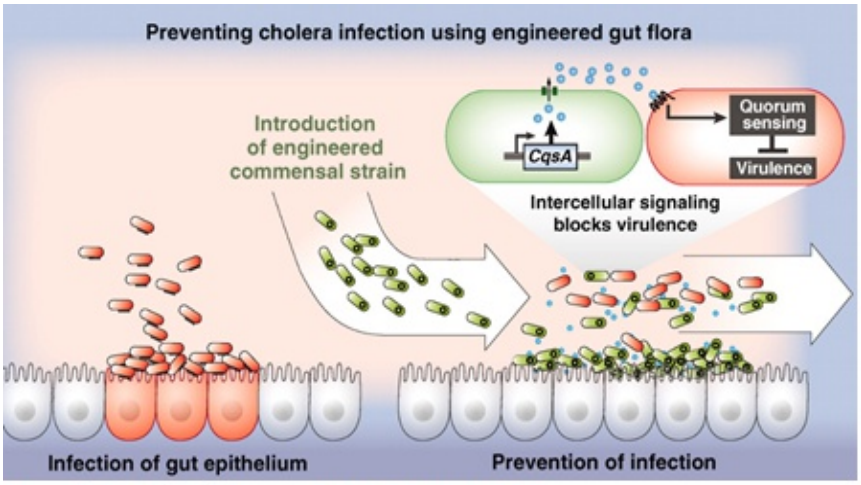

Figure 5: Duan, Faping, and John C March, 2010

These findings suggest that similar approaches can be used to prevent infectious diseases or provide therapeutic molecules. These applications can be further developed by placing the expression of therapeutic molecules under the control of sensors that detect certain conditions and be turned on or tuned as needed.

Treating Bacterial Infections

Before antibiotics, bacteriophages were used to fight bacterial infections[19]. Now with antibiotic resistance becoming a concern, bacteriophages are becoming an increasingly important alternative to antibiotics. For example, researchers engineered lytic bacteriophage T7 to constitutively express dispersin B (DspB), an enzyme that hydrolyzes an important adhesion molecule required for biofilm formation[20]. Biofilms are surface-associated bacterial communities in a hydrated matrix of extracellular polymers composed of polysaccharides, proteins, and lipids[21]. This extracellular matrix is vital in many types of bacteria to resist and evade the immune system as well as antibiotics. By targeting the biofilm, researchers were able to develop a bacteriophage that killed the bacteria. The bacteriophage operates in a “two-pronged attack.” The phage first lyses the infected bacterial cells and releases DspB along with more copies of

itself. The DspB then begins to degrade the biofilm matrix and expose the unprotected bacteria to the phage resulting in a cyclic process that kills 99.997% of the bacterial cells.

In a follow up study by Collins and colleagues, M13 phage was engineered to enhance the efficacy of antibiotics in a phage-drug combination therapy by acting as a synthetic adjuvant[22]. They did this by disrupting bacterial gene networks that regulate antibiotic defense mechanisms, such as the SOS network in E. *coli*. The SOS network is activated in response to bacterial DNA damage caused by antibiotics. Nonlytic M13 phage was engineered to inhibit this response by overexpressing lexA3, a repressor of the SOS pathway[22]. In vitro treatment with the engineered phage and antibiotics resulted in a 5000-fold increase in the killing of resistant bacteria compared to treatment with antibiotic alone. In an animal study, treatment with the phage resulted in 80% survival rate in *E. coli* infected mice, compared to 20% with only antibiotics. Phage therapy is currently being revisited in several clinical trials and is becoming increasingly more important as bacteria become multi-drug resistant (Figure 6)[23].

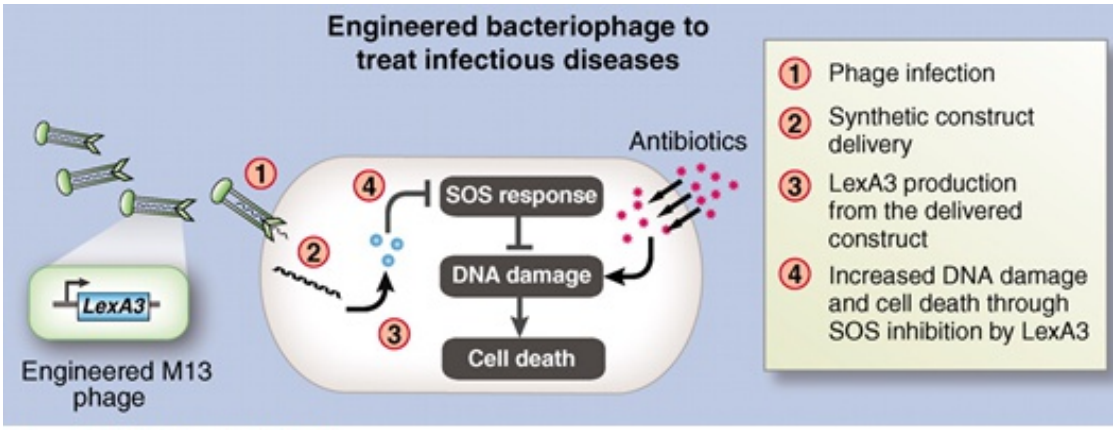

Figure 6: Collins et al. 2011

Targeted Cancer Treatment

Current cancer therapies focus on non-selective cell killing such as radiation and chemotherapy that are harmful to the patient. Synthetic biology offers alternative strategies for cancer treatments that enable cancer treatments to be more specific and targeted only towards cancerous cells.

Two studies have made progress to this extent. In one study, researchers engineered bacteria to invade cancer cells in hypoxic environments—typical of cancer tissues. *E. coli* were engineered to express the invasin (inv) adhesion protein from *Yersinia pseudotuberculosis*. Invasin tightly binds B1 integrin receptors and induces uptake in mammalian cells. By placing invasin expression under the control of anaerobically induced formate dehydrogenase promoter, the bacteria only invaded mammalian cell cultures in low oxygen environments[24]. This method can be further developed by coupling the invasion of cancerous cells with lethal payloads.

In another related study researchers engineered bacteria to produce a short hairpin RNA (shRNA) that targets the gene CTNNB1, which is overexpressed in many colon cancers[25]. The shRNA knocks down the expression of CTNNB1 by binding to CTNNB1 mRNA transcripts and inducing mRNA cleavage. This delivery system also employs invasin to induce uptake by cancerous cells and listeriolysin O gene HylA, which encodes two bacterial factors that are needed for successful transfer of the shRNAs into mammalian cells. Intravenous administration of the engineered *E. coli* into immune-deficient mice with xenografted human colon cancer cells resulted in a significant knockdown of the tumor causing gene.

Future Outlook

The potential of synthetic biology in medicine is promising, with applications in drug and vaccine development, therapeutic microbiome engineering, fighting bacterial infections, and targeted cancer treatment only scratching the surface. The benefits to human health are clear, however, the technical challenges ahead are enormous. Compared to the disruptive new industries of the 20th century such as automobiles and computers, biological systems are infinitely more complex and much less predictable[26]. In a 1974 panel discussion Geneticist Waclaw Szybalski described the future of biological innovation, “Up to now we are working on the descriptive phase of molecular biology…but the real challenge will start when we enter the synthetic phase of research in our field. We will then devise new control elements and add these new modules to the existing genomes or build up wholly new genomes. This would be a field

with an unlimited expansion potential.[27]" The scientific foundations and engineering processes have yet to be developed to allow synthetic biology based applications to become a clinical reality. But when they do reach the clinic, synthetic biology is poised to revolutionize healthcare in the twenty-first century.

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