Biomedical Synthetic Biology: An Overview for Physicians

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ABSTRACT: Synthetic biology is a relatively new field of biological research and development that focuses on the engineering of genetic molecular machines with a specific predefined function. Plainly put, the newly engineered organism functions as a machine. It can process information, manufacture, heal and even diagnose. We just have to engineer it to do so. The famous quote "Biology is the nanotechnology that works" is currently being put to the test on a worldwide scale. The application of these machines is theoretically boundless. In laboratories worldwide synthetic biology technologies are being rationally designed to assist in diagnosis or disrupt disease mechanisms. In the not too distant future they are expected to reach the clinical setting. This new field should be distinguished from classic genetic engineering. The latter researches naturally found DNA segments via cloning. It is weakly associated with engineering. Synthetic biology focuses on the engineering of molecular biological machines for the benefit of mankind. This is done via synthetic (computer printed) DNA sequences, man-designed or altered *in silico*. In this article I will briefly introduce synthetic biology, elaborate on the Biobrick Foundation as an independent fast-growing synthetic biology-sharing movement, and report on selected developing applications for medicine.

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Recombinant DNA refers to man-created DNA sequences that are not found in natural biological organisms. Synthetic DNA is a type of recombinant DNA, designed *in silico* and manufactured by special DNA printers. Using cloning, virtually any synthetic DNA sequence may be created and introduced into a wide range of living organisms. The most common application of this technology is biological research, yet its contribution to our everyday lives cannot be overlooked. In medicine, recombinant DNA technologies have been used to design and manufacture human insulin, human growth hormone, hepatitis B vaccination and more.

Physicians should be aware of synthetic biology as an emerging technology with a potential impact on medicine

Synthetic biology is a relatively new field of biological research and development. It makes use of recombinant DNA to engineer biological machines not found in nature from living organisms by rewriting DNA code. Biological machines have several advantages over the non-biological: they already work in a nanotechnology scale environment, the hardware (cells) is developed and only the software (DNA code) needs to be altered, and they are cheap to produce, store and maintain. They also have several disadvantages, as will be discussed.

The term "parts" is used in synthetic biology to describe a functional DNA segment. Its function may be derived from the DNA's chemical properties or from the coded RNA or protein products. Many basic parts are assembled *in vitro* into larger parts. These large parts, termed "systems," are rationally designed to conduct complex molecular functions when they operate inside living cells.

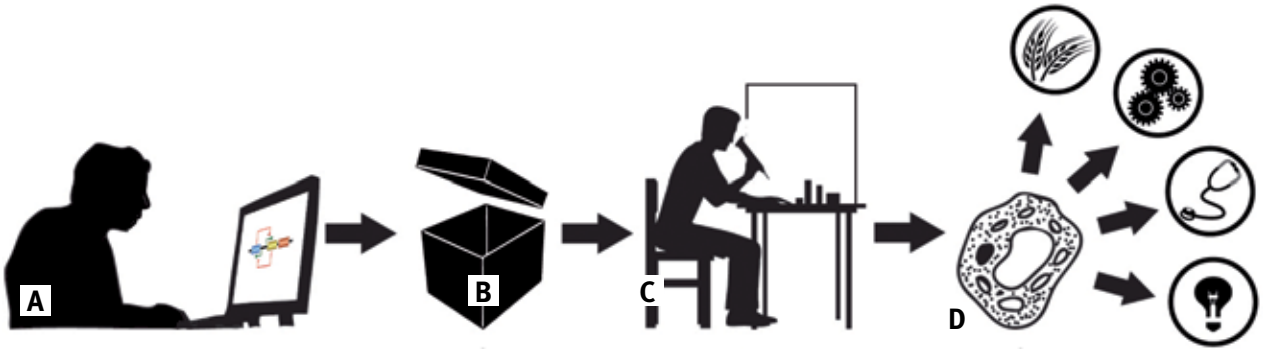
Synthetic systems require a working framework, which is termed "chassis." Chassis are usually, but not limited to, bacteria or cells with simple genomes that are receptive to foreign DNA. Viral phages are also used as chassis.

A general outline for synthesizing new biological systems is as follows [Figure 1]: parts and their interaction are designed *in silico*, synthetic DNA coding for parts is manufactured, parts are assembled into systems *in vitro* via cloning, and systems are transfected into a chassis in a laboratory. Inside the chassis the DNA parts are expressed by host proteins. The new RNA and proteins interact together to form the new "machine" which functions inside the chassis. The newly engineered organism now functions as a machine.

Two mechanical simplified gene networks designed in the previous decade prompted molecular biologists to direct their attention to the new possibilities of biological engineering [1,2]. Other lab groups followed and developed increasingly sophisticated synthetic systems. Over a short period, synthetic biology emerged as an independent concept and field. Whole synthetic cells with entire synthetic genomes were a milestone that caught the eye of the lay media in 2010 [3]. These landmark experiments reveal the initial intentions of synthetic biology: to rationally research simple cellular dynamics. However, the trend is shifting towards applicable systems designed for use in various industries.

We are now entering a post-genomic era where a database exists for every known aspect of genes and their interactions in thousands of possible organisms. The past 50 years of molecular biology research have delivered an immense amount of data

Figure 1. Outlined scheme for synthetic biology. **[A]** Synthetic biological systems are designed *in silico* and DNA is ordered online. **[B]** Computer-printed DNA arrives from an outsourcing company. **[C]** Assembly is performed followed by transfecting the new system into a chassis. **[D]** The chassis with the working system is now a working machine. It is put to use in an endpoint industry such as food, manufacturing, biomedicine or energy



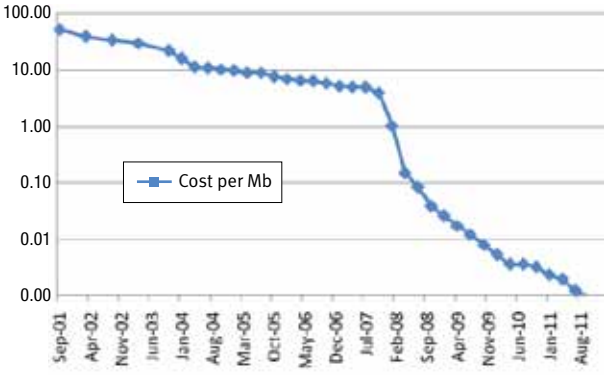
and a very large cache of well-characterized parts for use in synthetic biology.

Biomedical research continues to answer complex questions about the nature of cellular life, disease and treatment. In the meantime synthetic biologists are engineering new diagnostics, therapeutic and research techniques to bridge challenges in current or future medicine. Challenges such as cancer treatment, vaccine development, malaria eradication, antibiotic resistance and gene therapy are already the focus of synthetic biology teams globally. In a few years they are expected to reach the clinical setting.

DNA READING AND WRITING (CLONING)

Synthetic biology depends largely on the availability of cloning technologies. These technologies have undergone spectacular development over the past decades, both in diversity and in decreasing cost [Figure 2]. Examples of such technologies include: *in silico* DNA design, automated sequencing, synthetic DNA printers, and more. Cloning will eventually be completely mechanized or outsourced. This will allow synthetic biologists to focus on the creative aspect of biological system engineering.

Figure 2. The cost of DNA base pair sequencing (per million bases) in U.S. dollars, as a function of time, over the past decade. Note the Y axis is logarithmic. Source: National Human Genome Research Institute, USA



ENGINEERING APPROACHES

Engineering approaches used by synthetic biology allow easier and faster design, construction and assembly of biological systems. Key approaches include abstraction, modularity of systems and standardization. In abstraction, concepts are combined or transformed into higher concepts in order to hide information and manage complexity. Abstraction relieves end-users of the knowledge required of engineers. An appropriate analogy would be the billions of computer users worldwide who do not understand software programming code.

Modularity is the degree to which components may be separated and recombined, mixed and matched in a variety of configurations. This allows component interaction and resource exchange (such as energy or data). Modularity is important for system hierarchical design. Standardization is the establishment of uniform technical criteria, methods or interfaces. Standards in synthetic biology promote parts sharing and the establishment of official part collections.

THE BIOBRICK FOUNDATION

The Biobrick Foundation is an independent synthetic biology group based at Massachusetts Institute of Technology and founded in 2003. Its goal is to promote biotechnology for the public benefit. The foundation's key pioneers came from the computer software industry. They adapted strategies from their own industry to life science engineering, such as an open-source approach. They created the Biobrick assembly standard for parts, which relies heavily on engineering approaches such as mentioned above.

The Biobrick standard is a collection of engineering protocols for DNA segments officially referred to as "request for comments" (RFC). RFC number 10, draft standard for

Biobrick biological parts, is of special interest. It describes the DNA sequence necessities of small parts in order for them to undergo parallel assembly.

ASSEMBLY

Assembly is an important step in the engineering of every machine, including biological. Classic DNA assembly, employed by genetic engineers, involves adding one DNA segment at a time to a growing sequence of DNA. Biobrick's parallel assembly places emphasis on engineering principles such as mentioned above to simplify and enhance the assembly process [4]. It involves the assembly of multiple DNA segments simultaneously using specific adaptor regions designed for this purpose. This protocol has a more than 97% chance of selecting only the desired end-product from other assembly byproducts.

This protocol has several advantages: a) it reduces the number of reactions needed for assembly in a logarithmic fashion, and consequently, the error rate as well; b) the simplicity of this model attracts amateur biologists to synthetic system design; and c) a common adaptor enhances part-sharing between labs. The protocol also has several disadvantages: a) more than one reaction is needed for almost every assembly; and b) every reaction step creates small DNA scars that mutate the DNA segment.

The Gibson assembly is an alternative DNA parallel assembly method. Invented in 2009 by Daniel Gibson [5], this method assembles multiple sequences of DNA with overlapping end-sequences at their joining point. It requires a single isothermal reaction and does not create a scar. The disadvantage of Gibson's assembly is the need for custom-fit reaction primers for every assembly. This disadvantage necessitates advanced planning and is more costly.

The Biobrick standard offers a concise and simple model for sharing and engineering synthetic systems

THE REGISTRY OF STANDARD BIOLOGICAL PARTS (HTTP://PARTSREGISTRY.ORG)

This is an online open-source part catalogue that conforms to the Biobrick standard. It contains roughly 3500 parts that have been uploaded by a variety of users and groups, all available to order online. Many of these are high quality Biobrick parts that are relevant, tested and rated. Users of the parts include academic and commercial labs, established scientists and student teams.

The International Genetically Engineered Machine student competition is an annual event held at MIT [6]. Each participating team presents a project and parts with a certain industrial track (new applications, foundational advancements, information processing, software tools, environment, energy, manufacturing or biomedicine) from which winners are selected. The teams participating in iGEM have designed many innovative useful parts, and added them to the registry.

MIT = Massachusetts Institute of Technology
iGEM = International Genetically Engineered Machine

The utility of the parts registry is becoming apparent due to the re-use of old parts in new projects. The iGEM student competition is continuously expanding, with 214 registered international lab teams in 2013. While many iGEM parts and projects do not work as planned, the large contribution to synthetic biology in such a short time cannot be overlooked.

Sharing and standardization drive open-source networks to expand. Through the Biobrick model, synthetic biology has the possibility of reaching a new multidisciplinary audience. This audience has designed creative new parts and systems despite their amateur knowledge of biology. In the "do-it-yourself biology" vision, users will swap and alter biological parts to construct custom-fit systems, in a manner similar to that of the computer hardware revolution of the 1970s. A fast and reasonable way to achieve this is by means of standardized synthetic biology, as suggested by the Biobrick model.

SYNTHETIC BIOLOGY BIOMEDICAL APPLICATIONS UNDER DEVELOPMENT

Some synthetic biology labs have chosen to develop parts and systems for use in the biomedical and clinical setting. Applications are being developed for a variety of clinical fields, such as oncology treatments, regenerative medicine, infectious disease control, drug delivery systems and many more. Most of these are complex systems and parts that are beyond the scope of this article. This section highlights a few examples of developing biomedical application in synthetic biology, while presenting the field's current clinical reach. Most synthetic biology labs and teams do not focus their efforts on biomedical applications. However, they are designing new molecular functions that may prove useful in the future of biomedical synthetic biology as well.

SYNTHETIC APPLICATIONS RELATED TO BACTERIAL DISEASE MANAGEMENT VIA PHAGE THERAPY

In the era of hospital and community-acquired resistant infections, physicians are searching for new tools to augment or even replace current antibiotics. Synthetic biology might be able to deliver these tools.

Phage therapy refers to the therapeutic use of naturally occurring bacteriophages to treat bacterial infections. It was recognized as a clinical potential in the early 1940s; since then, phages were extensively researched and used in the former Soviet Union [7]. Currently they are not approved for use in countries other than Georgia. The concept is being revisited by synthetic biology. Phages containing synthetic systems may be used to prevent hospital-acquired infection and treat drug-resistant bacterial infections.

In one study, phages were engineered to degrade bacterial biofilms and cause the bacteria to lyse. This was followed by rapid replication of the phage and reinfection of the bacteria.

The resulting cyclic process eventually removed 99.997% of bacterial cells in treated biofilms [8]. Compared to control phages, the modified phages were twice as efficacious in degrading bacterial biofilms. These studies suggest the potential of synthetic phages to reduce the incidence of catheter-related infections.

In another study phages were designed to disrupt bacterial DNA repair mechanisms [9], a strategy thought to augment quinolones. In animal studies, co-treatment with engineered phage and ofloxacin resulted in an 80% survival rate in *Escherichia coli*-infected mice, compared to 20% with antibiotic treatment alone. The phage therapy also augmented the bactericidal effect of other antibiotics (e.g., aminoglycosides and beta-lactams).

SYNTHETIC APPLICATIONS RELATED TO VACCINE DEVELOPMENT

Synthetic biologists have attempted to address challenges in vaccine design. Some examples of challenges include the risk of using attenuated pathogens as vaccines and difficulties in dictating a specific immune response to a vaccine.

The use of liposomes, artificially made lipid bilayer vesicles, for the delivery of synthetic systems might be one such tool. Some liposomes have the advantage of containing a special immune privileged microenvironment. Synthetic liposomes have been engineered to produce antigen protein in mice *in vivo* [10]. These vesicles contain translation machinery proteins and a DNA template that codes for the desired antigen. The DNA template is translated into its protein product inside the liposome. The protein product is then exported from the liposome and acts as an antigen in the extracellular space. Mice treated with the synthetic liposome showed a higher humoral immune response as compared with control vaccines. This system can be easily altered for other antigens by simply changing the DNA template, and it carries no risk of infection by attenuated pathogens.

Algae-based oral staphylococcal vaccination is a eukaryotic chassis-based vaccine. In one study algae chloroplasts were engineered to express staphylococcal antigens [11]. After 5 weeks of feeding mice with the algae-expressing synthetic systems, the researchers noted specific immune responses in the treated mice. Up to 80% of these mice were protected from staphylococcal lethal dose injections.

The iGEM grand winner of 2008, the team from Slovenia, attempted a different approach to vaccine design [12]. They linked *Helicobacter pylori* components to toll-like receptors of the innate immune response. This guided *H. pylori* proteins to relevant compartments within the immune cell, causing optimal innate and acquired immune response. The vaccine has been thoroughly characterized *in vivo* and *in vitro*, exhibiting a substantial antibody response.

DEPLOYMENT OF SYNTHETIC SYSTEMS IN THE HUMAN MICROBIOME

The human microbiome, the totality of microorganisms associated with the human body, is increasingly gaining attention as a possible niche for synthetic system transplantation. The human body contains over ten times more microbial cells than human cells. Because these organisms are well tolerated, they are potentially excellent vectors for deploying synthetic systems.

Commensal bacteria strains have been engineered to secrete key molecules for potential disease treatment. Some examples include insulinotropic proteins for diabetes [13], a human immunodeficiency virus fusion inhibitor peptide for prevention of HIV infection [14], and interleukin-2 for immunotherapy [15].

The microbiome could also theoretically be used to alter simple metabolic pathways. Removal of unwanted metabolites by synthetic systems could potentially treat storage diseases. One study attempted mammalian system design [16] to augment uric acid homeostasis *in vivo*. This system could potentially be used to prevent tumor lysis syndrome and gout. The system produces the enzyme urate oxidase upon activation, eliminating the uric acid. These synthetic systems were implanted in urate oxidase-deficient transgenic mice with high levels of blood uric acid. Following implantation their urate concentrations changed to subpathological levels and reduced uric acid crystal deposits in the kidneys.

SYNTHETIC APPLICATIONS RELATED TO ENVIRONMENTAL PARASITIC CONTROL

Parasites, which are a frequent cause of morbidity in developing countries, are a growing focus among synthetic biologists. The iGEM 2010 grand finalist team of Imperial College London engineered a sensor for rapid detection of a range of different parasites. They designed and modified *Bacillus subtilis* to give a clearly visible color readout on detecting Schistosoma parasite antigen in water, a threat that affects 200 million people worldwide. This sensor may be used to prevent schistosomal infections or used as an environmental tool for mapping their spread.

TECHNICAL CHALLENGES FOR SYNTHETIC BIOLOGY

Synthetic biology is popularized in the layman media with a certain hype, characterizing a genetic sequence and its product (proteins and RNA) that performs a certain function, and combining many of these parts into a system to achieve more complex function. The new system is then inserted into living cells to activate the machine. In truth, biological system design is a complex process and one that carries technical and ethical challenges, most beyond the scope of this article.

Classic biological research denudes natural biological systems into isolated components and examines them thoroughly. Here we reintroduce engineered components into an already complex and evolving environment. Thus, a certain error potential and unpredictability are to be expected. For

HIV = human immunodeficiency virus

these reasons, most synthetic systems have remained of low complexity and have been utilized in a bacteria chassis. Therapeutic challenges will eventually require synthetic systems to perform complicated tasks, as well as work in the mammalian cell chassis.

Synthetic biology faces many other technical challenges. DNA mutations, which arise in parts over time, will contribute to their eventual decay. Parts and their intracellular modifications are not necessarily compatible between species. Systems have unpredictable environment interactions, a factor that may cause side effects [17].

Though clinical synthetic biology has the potential to cause a huge impact, its clinical use will have to undergo the same ethical, legal and scientific review as any other developing therapy. Clearly, it will be some time before these technologies can harness its full clinical impact. The examples described above provide insight into the field's exciting potential for helping to prevent and treat disease.

OUTLOOK

The isolation of restriction enzymes in the early 1970s opened the door for recombinant DNA technology. It was then postulated that cloning would eventually become inexpensive and widespread, allowing for the creation of synthetic biology, or biology that does not exist in nature. Today, after 35 years of cloning, these technologies are indeed not costly. One striking example is the sequencing of the human genome, which cost less than US$ 10,000 as of 2012.

The field initially arose from the combined efforts and insights of computer software engineers whose backgrounds dictated the early directions of synthetic biology. Their goal was to use biology to rebuild biology. The open-source model that is employed by the Biobrick Foundation is currently a successful sharing modality for synthetic systems. It has resulted in the recruitment of a whole generation of biologists to its cause.

Synthetic biology is gaining worldwide academic legitimacy. New dedicated labs and centers are being founded worldwide, such as at the Imperial College London, Harvard University's Wyss Institute in Boston, and more. Although synthetic biology is in its infancy, pioneers are taking initial steps toward developing new biomedical therapies. When the field becomes better integrated with health care practitioners it may start to reach its full clinical potential [18]. Today's synthetic biology is compared by many to the computer industry in the mid-1900s. Then, computer science was viewed as a complex, analytical time-consuming discipline that was more science than technology. Until recently synthetic biology was described in the same way. In truth, synthetic biology may be the beginning of a true nanotechnological industrial revolution, and medicine is expected to be impacted.

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"If you write to impress it will always be bad, but if you write to express it will be good"

Thornton Wilder (1897-1975), American playwright and novelist, and laureate of three Pulitzer Prizes

"Every increased possession loads us with new weariness"

John Ruskin (1819-1900), English art critic of the Victorian era, also an art patron, watercolorist, a prominent social thinker and philanthropist. In all of his writing, he emphasized the connections between nature, art and society