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Opinion

# Engineering Life through Synthetic Biology

Paras Chopra[a,*] and Akhil Kamma[b]

[a] *Delhi College of Engineering, Bawana Road, New Delhi-110042, India*
*Tel.: +91 01127563421; E-mail: paras.chopra@bt.dce.edu*
[b] *Delhi College of Engineering, Bawana Road, New Delhi-110042, India*
*Tel.: +91 0 9873007214; E-mail: akhil.kamma@gmail.com*

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**ABSTRACT:** Synthetic Biology is a field involving synthesis of novel biological systems which are not generally found in nature. It has brought a new paradigm in science as it has enabled scientists to create life from the scratch, hence helping better understand the principles of biology. The viability of living organisms that use unnatural molecules is also being explored. Unconventional projects such as DNA playing tic-tac-toe, bacterial photographic film, etc. are taking biology to its extremes. The field holds a promise for mass production of cheap drugs and programming bacteria to seek-and-destroy tumors in the body. However, the complexity of biological systems make the field a challenging one. In addition to this, there are other major technical and ethical challenges which need to be addressed before the field realizes its true potential.

**KEYWORDS:** Synthetic biology, life engineering, bioengineering

## INTRODUCTION

Synthetic Biology is an art of engineering new biological systems that don't exist in nature. It is also redesigning existing systems so as to understand their underlying mechanisms.

At a basic level, Synthetic Biology is breaking biological processes such as the production of a protein from a gene and then stitching the components to build a desired system that performs a particular function like oscillators which oscillate between producing a protein or not.

Synthetic Biology attempts to create living systems from the scratch and then endowing these systems with new and novel functions. The molecules used in these systems might be naturally occurring or artificially synthesized. The mode of production is not synthetic because the resulting compound is still produced biologically. The term synthetic comes from the fact that the compound is produced from an organism with a genetic code that is not ordinarily found in nature.

The term 'Synthetic Biology' was coined by Barbara Hobom while describing genetically engineered bacteria [1,2]. However, the heavy emphasis on foundational technologies is an aspect which distinguishes it from genetic engineering.

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[*] Corresponding author.

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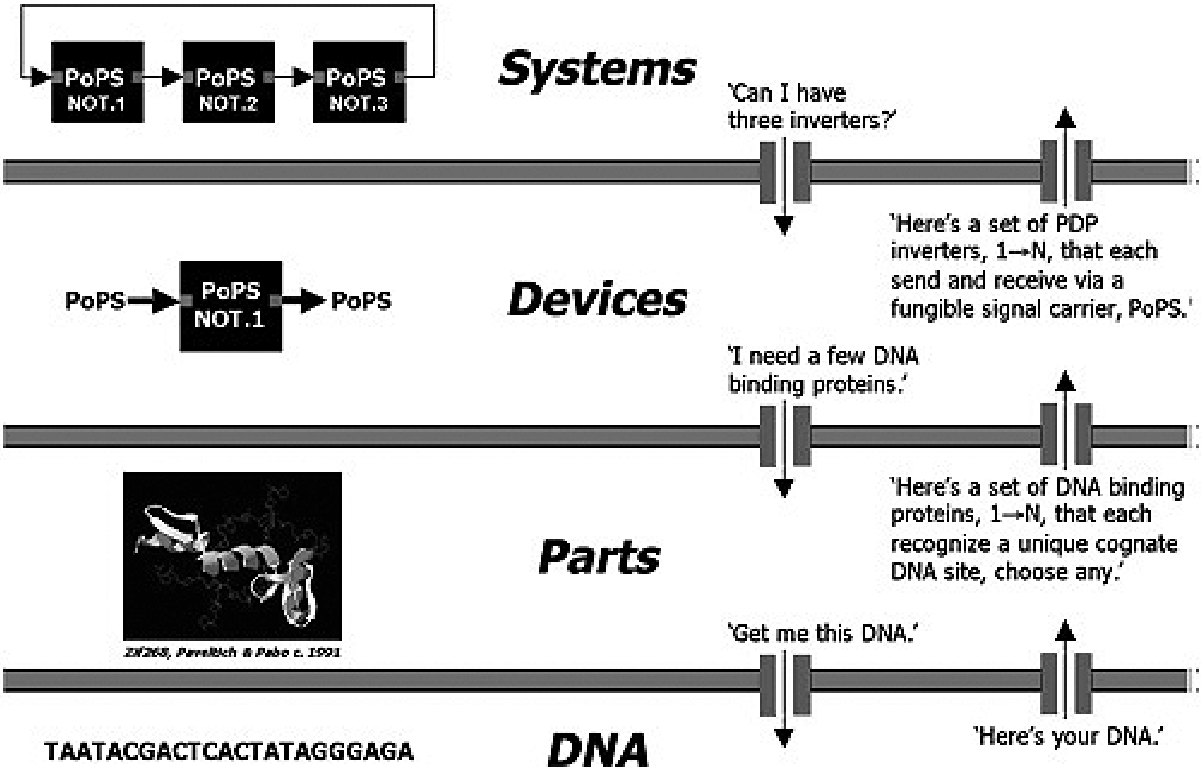

Fig. 1. Possible abstraction hierarchy in biological systems (http://openwetware.org/wiki/Image:AbstractionHierarchy.jpg; courtesy of Drew Endy [3]).

## APPROACHES TO SYNTHETIC BIOLOGY

There can be various approaches to Synthetic Biology. The approaches in brief are:

### *Engineering of biological systems*

The attempt to synthesize biological components which can be assembled together to create biological circuits which behave in predictable way. The biological components may be interchangeable in the circuit. Thus, it is an attempt to bring existing concepts such as standardization of components, decoupling of problems and abstraction of information from engineering realm to biology [3]. Figure 1 pictorially depicts a possible heirchacy in future biological systems.

### *Redesigning life*

By constructing biological systems, gaps in our current understanding of biology can be revealed by observing differences in observed and predicted behavior. In other words, rewriting the complete genome from the scratch will enable us to understand biology in a better way. In addition to this, minimal living systems can be developed through this approach, which can then serve as entities that can be augmented for specific applications.

### *Creating alternative life*

This approach mainly involves the use of unnatural molecules in living systems. The current living systems are composed of molecules such as DNA, RNA, proteins, etc. But, there are certain unnatural molecules, which can perform the same function as these molecules [2].

Taking into account all above approaches, we define Synthetic Biology as a field involving synthesis of novel biological systems which are not generally found in nature. Here we define 'systems' abstractly so as to accommodate the various diverse but overlapping approaches mentioned above.

## CURRENT INITIATIVES BY THE COMMUNITY

Researchers worldwide are using the above mentioned approaches to explore the scope of Synthetic Biology and simultaneously develop foundational tools and techniques in this field. In this section, we discuss various initiatives taken by the researchers.

Biological components are independent biological parts which can be assembled together to function in a predefined manner. These can be compared to electronic components such as resistors and capacitors which are used in an electrical circuit. An important point to note here is that these biological components must work on some sort of standard and thus must be interchangeable. One such standard which has been proposed by researchers is Polymerase per Second (PoPS). PoPS is the flow of RNA polymerase over DNA. It is analogous to electric current which is flow over electrons in the conductor. Since the nature of DNA transcription is independent of the underlying DNA sequence, PoPS serves as a standard for biological systems. For this reason, we believe the PoPS can serve as a *resonably good standard* in this field.

Thanks to researches being done worldwide, a large number of biological parts are being produced. The Registry of Standard Biological parts (http://parts.mit.edu/) has been setup by MIT as an attempt to standardize these parts. An initiative like this certainly aims to bring an engineering approach to biology. Moreover, competitions such as Intercollegiate Genetically Engineered Machine competition (iGEM) foster interest of young scientists in this field and also help in devising experiments related to Synthetic Biology such as oscillators, counters, bacterial switches, etc. (http://parts.mit.edu/). Although the projects have not been found to be successful to a large extent but this competition is certainly strengthening the foundation of the field.

An example of an attempt to redesign life is shown by Drew Endy of MIT who is rewriting the genetic code of bacteriophage T7 [4]. This virus will be different from its natural counterpart as it lacks overlapping genes. This artificially synthesized virus will greatly aid researchers who study it because it will have distinctly spaced genes.

Yet another initiative in this field is the synthesis of artificial biological/chemical systems, which support Darwinian evolution [2]. Thus, it is an attempt to bring concepts such as inheritance, genetics, evolution and possibly self-reproduction to artificial systems. With the cost of DNA synthesis dropping rapidly, the goal seems highly probable.

Current applications which use this approach of artificial genetics include diagnostic tools such as Bayer's branched DNA assay [2]. It has been estimated that close to 40,000 patients infected by HIV and hepatitis viruses rely on Bayer's branched DNA assay for their care.

There are also some toy-projects being pursued by researchers. It must be noted that even though these projects are said to be toy or fun projects, they are no less important. They hold immense promise for the development of future technologies and sciences. Indeed, they can be viewed as proof-of-concept projects. Following are few of the examples of such projects:

Fig. 2. A photosensitive bacterial biofilm made during 2004 Synthetic Biology competition. See [7] for details of the process of the synthesis of photosensitive biofilms. (http://en.wikipedia.org/wiki/Image:UT_HelloWorld.jpg; courtesy of Jeff Tabor and Randy Rettberg).

1. DNA plays tic-tac-toe [5]: Even though it is not an excellent application but it is good enough to demonstrate that logic can be encoded into one of the basic components of life i.e. DNA. If we can scale up this DNA computation in future via Synthetic Biology, then massively parallel computers could be made that would be very cheap.
2. Biological counters: Pamela Silver of Harvard University is trying to make a biological counter for yeasts [4] so that every time the yeast divide, a gene encoding for fluorescent protein gets activated, hence the cell shows fluorescence which can be detected. Currently, her modest goal is to count up to two. The counter will help her to study how cells age.
3. Bacterial image production (Fig. 2): Researchers have manipulated bacteria so as to convert a lawn of bacteria into a biological film [6]. The projection of a pattern light lets bacteria produce a two dimensional picture of a very high definition (about 100 megapixels per square inch). They propose that this system can serve the purpose of printing biological materials and could also be used for investigating signaling pathways in future.

## APPLICATIONS OF SYNTHETIC BIOLOGY

Synthetic Biology has the potential to make the process of development of an organism comprehensible to us as well as unlock many of the mysteries of biology with possible applications in medicine and energy production. It is believed that Synthetic Biology shares the potential, together with Nanotechnology and Artificial Intelligence to generate new entities [7], which can reproduce and evolve at will. These entities can then be used to solve problems not possible by a human mind.

Few possible applications of Synthetic Biology are discussed below:

### *Cheap and environmentally friendly production of medicine from microorganisms*

By combining genes to create chemical factories within microorganisms, synthetic biologists hope to produce new drugs to fight disease, combat bioterror agents, and produce existing drugs without depleting our natural resources. A good example to illustrate this is the production of the anti-malarial drug precursor *artemisinin*. The drug produced by a wormwood plant that grows in Southeast Asian mangrove swamps is too expensive in developing countries where malarial bacteria is growing resistant to affordable treatments. By inserting genes from three separate organisms into *E. coli*, synthetic biologists have created a bacterial strain that can produce the precursor to artemisinin [4]. *Artemisinin* produced this way costs about half as much as it costed earlier.

In addition to the cost advantage, batches of the drug are more consistent as far as availibility is concerned. Production of the drug would not be subject to political instability or deforestation, a problem in tropical nations, from which many of the latest drugs come. Expanding on this work, synthetic biologists hope to use this same technique to mimic a chemical pathway found in a medicinal tree of the Philippines called the Mamala to develop a drug that fights HIV.

It can be argued that the techniques employed by the researchers above are common in Molecular Biology and that the technique is nothing new but traditional Genetic Engineering. There is, however, a subtle difference between traditional Genetic Engineering and Synthetic Biology. Unlike Genetic Engineering, which involves transfer of a gene from one organism to another via hit-and-trial method, Synthetic Biology involves design of a genetic circuit which can have components from many distinct species. Genetic Engineering is a misnomer because there is hardly any engineering involved in the field. Synthetic Biology, however, relies heavily on the philosophy behind engineering such as previously mentioned concepts of standardization and abstraction.

### *Energy production*

Synthetic biologists could use the molecular machinery in microorganisms like *Bacillus subtilis* to efficiently capture the energy stored in plant biopolymers like cellulose. These approaches could lead to microorganisms producing hydrogen, to improve the fixation of carbon dioxide or nitrogen and efficiently converting sunlight energy into other chemical forms.

### *Bioremediation*

Synthetic biologists are trying to engineer microorganisms to remediate some of the most hazardous environmental contaminants, including heavy metals, and nerve agents like sarin. Such organisms have enormous potential for decontaminating hazardous waste spills and treating byproducts from nuclear energy and disposal sites.

### *Biological threat detection and decontamination*

Synthetic Biology could eventually lead to the development of new and novel techniques to treat deadly diseases. A new kind of biological cells are being engineered that would swim to tumors inside our bodies and destroy them. Arkin and Chris Voigt of University Of California, San Francisco are assembling biological parts which may help *E. coli* fight cancer in future [8].

### *Programming cells for information processing, communication and gene regulation*

Synthetic gene networks will also enable us to develop new logical forms of cellular control, which could have important applications in functional genomics, nanotechnology, and cell therapy. Other applications include tissue engineering, molecular fabrication of biomaterials and nanostructures, biosensing, and improved understanding of the operating principles that govern living organisms. Our aim is to program cell behavior as easily as we program a computer. An example is a genetic toggle switch interfaced with some part of the cell's natural regulatory circuitry like the SOS signaling pathway responding to DNA damage through appropriately designed input and output interfaces.

Another example of information processing is the DNA playing tic-tac-toe mentioned earlier.

## Obstacles to success

Many problems confront Synthetic Biology before it goes into mainstream. These problems come in diverse colors; ranging from being ethical to technical.

### *Synthetic Biology may spawn a new era of hackers*

It has been proposed that Synthetic Biology can lead to the accidental or deliberate creation of pathogenic biological components. To a certain extent it is true as the designing and engineering of molecular machines could be made easier by reducing the molecular biology of the cell to a list of standard modules with predictable behavior. Just as a circuit designer need not be an expert in silicon physics and manufacturing processes, the future ‘biodesigner’ will not need a detailed knowledge of biochemistry to effectively create complex biochemical machines [9]. However, just like any other field, Synthetic Biology needs a regulatory body which acts to prevent the creation or spreading of artificial pathogenic biological systems.

### *Self-replicating nature of biological systems*

Some observers believe a ban on self-replication as the only way to head off the threat posed by biosynthesized systems (like some engineered molecular system running rampant). But it might just be too late for something like that. And moreover the self-replicating nature of biological systems is a built-in manufacturing system, although one that is prone to variations in the form of mutations. In addition to this, the biosynthesized systems may also not survive in the wild because of their special nutritional and environmental needs.

### *Concerns about uncontrolled spreading of genetically modified organisms*

Dangerous organisms could be created, deliberately or by accident, which can go wild and create havoc. The rapid pace of technology growth may seem to make us believe that the synthetic genome work for genetically modifying an organism is likely to lead to a more rapid development of organisms with new capabilities. But, this is not entirely true as measures taken by the Synthetic Biology community will prevent uncontrolled spreading of engineered organisms. Researchers are proposing to include a DNA watermark in engineering biological systems so that their spread can be tracked.

### *Ethical considerations*

The technology of Synthetic Biology provides a new set of tools. Any ethical challenges come from the way we use the tools and not from the tools themselves. People might object to new developments with a view that humans can’t play *God* as was seen in case of genetically modified crops. The issues raised by Synthetic Biology technology are similar, and any ethical concerns unique to this technology are not foreseen.

### *Expensive and unreliable research process*

Presently, Synthetic Biology is an expensive and unreliable research process. It is expensive because DNA synthesis is quite expensive. For example the bacteria *Mycoplasma genitalium* has the smallest genome out of all living cells [10]. It consists of just 517 genes, totaling to 580 kb. Taking the current average price of gene synthesis as $1 per base pair, it would take 5.8 million dollars to synthesize just a minimal living entity. So, currently, mass production of applications based on Synthetic Biology is not yet feasible through artificial DNA synthesis.

Synthetic Biology might prove unreliable because unlike components used in other engineering approaches, the behavior of biological components such as DNA, protein, etc. is affected even by slight modifications in the internal or external environment of the cell. Apart from the piece of machinery working for the application there are numerous other components, which are vital for the survival of living cells, and these may interfere with the normal functioning of genetic circuit, hence rendering the internal environment unpredictable.

Thus, Synthetic Biology has to cope with the inherent randomness inside the cell. Unreliability also arises because unlike electronics there are no wires in the cell. A signal meant for one part of biological system may affect the other part. For example, if a biological system consists of 3 different inverters, then the signal meant for $1^{\mathrm{st}}$ inverter may diffuse inside the cell and affect the $2^{\mathrm{nd}}$ and $3^{\mathrm{rd}}$ inverters directly. This may trigger inappropriate responses.

### *Biological systems are too complex*

There is a possibility that we do not know enough about biological systems and that they might be too difficult to engineer. Furthermore, it is possible that the designs of natural biological systems are not optimized by evolution for the purposes of human understanding and engineering. The lack of foundational technologies makes engineering of biological systems an even bigger problem by making it a research problem rather than an engineering problem [3]. Biological circuit engineers would still be unable to predict the precise behavior of even the simplest synthetic networks, a serious shortcoming and challenge for the design and construction of more sophisticated genetic circuitry in the future [11].

### *Unanticipated results with increasing gene number*

Difficulties arise as complex sets of genes are brought together. When extensively modifying the genetic composition of a microbial cell, the changes must be consistent with cell survival in the context of the desired biological niche.

### *Evolution*

On the one hand, we have the boon of self-reproduction of living cells, which may lower the costs of production of the biological systems. But on the other hand we have evolution. In the context of working efficiency of applications of Synthetic Biology, evolution is undesirable. This is because the mutations may render a genetic circuit un-operational very soon after it comes into existence. There are certain cases when mutations destroy the functionality of genetic circuits in as less as five hours [12]. Thus, it has become a major challenge for synthetic biologists to address the problem of evolution.

## GOALS THAT NEED TO BE ACHIEVED

### *Technical*

The first step to the proper implementation of Synthetic Biology is its standardization. Standard biochemical modules with standard inputs and outputs will have to be defined. This would be followed by the designing of some automated, highly parallel manufacturing system that can take a description of a system in terms of standard parts and crank out actual biological components [9].

Secondly we need to develop a certain limited set of predefined materials in a ready to use state and that behave as expected. This would include a set of rules that describe how these materials can be used in combination. Skilled individuals with a working knowledge and means to apply these rules would also be needed [3].

Our success also depends a great deal on the rate of DNA synthesis. Chemical reactions are prone to errors, and a major barrier that slows down DNA synthesis is the need to correct errors and verify the correctness of a molecule that can have hundreds of millions of base pairs.

We need to create disposable biological systems. The use of these disposable synthetic genomes would enable rapid modifications of biological system to minimize the effect of mutations as we can then dispose off mutated systems, allowing longer, more reliable production runs. Another approach could be the engineering of cells to attain and maintain lower mutation rates which can be obtained through a combination of systems modeling and directed laboratory evolution. This would go a long way in tackling the problem of mutations.

### *Moral and ethical*

Synthetic Biology needs to establish itself as a community effort that is safe and nurtures responsible practices and attitudes. For this, a code of ethics and standards need to be developed for biological engineering. Learning from gene therapy, we should imagine worst-case scenarios and protect against them. For example, full physical isolation and confined lab experiments on organisms should continue until we have data on a greater number of potential consequences – ecological and medical – of engineering such systems.

The saga of GM crops should be a learning lesson for us, biologists, and motivate us to carry out more experiments to establish that the field of Synthetic Biology can be made a safer one. This would mean farther global outreach and education in the field. The community needs to discuss the benefits of synthetic engineering to balance the necessary, but distracting, focus on risks.

Synthetic Biology needs to be pursued thoughtfully and responsibly. The Federal Government of the United States has established the National Scientific Advisory Board for Biosecurity (NSABB – http://www.biosecurityboard.gov/) to provide advice to Federal departments and agencies on ways to minimize the possibility of misuse of knowledge and technologies emanating from vitally important biological research. Governmental regulation is necessary but the best protection will be self-regulation by the community. The fact that the community is aware of the need for such self-regulation is proved by the declarations made during the Second International Meeting on Synthetic Biology (http://syntheticbiology.org/SB2Declaration.html).

On similar lines, George M. Church of Harvard Medical School suggested licensing for anyone designing systems with synthetic biological components [10] which might fend off the possibility of unintended side effects by maintaining a level of competency among the people in the profession, but would do little to prevent deliberate attempts by terrorists or hackers to create pathogens. Biological synthesis becomes fairly easy once the basic building blocks – the oligonucleotides – have been built. This means the regulation of the whole process could be centered on licensing and tracking them. The situation is similar to the nuclear industry, where difficult-to-produce fissionable material is closely tracked and stored in secure facilities. Something similar could be done for oligonucleotide production and distribution.

In addition to a code of professional ethics for synthetic biologists, we need to keep a watch so that they don't transgress. This requires not just laws, but also proper monitoring. Government experience in the surveillance of illegal drugs and hazardous materials would provide a big helping hand in this regard. In the commercial sector, monitoring systems could be used to reveal suspect activities, such as labs requesting DNA that is related to potentially harmful biological agents. The screening of precursor chemicals, nucleic acids, genes and designer cells against a pathogen database before its purchase is also a precaution that could be taken. However, automated monitoring will require a great deal of cooperation by manufacturers and international coordination. Discussions in this regard have already begun, including one supported by the Sloan Foundation. But any actions that would penalize the legitimate manufacturer or user are likely to backfire, and any laws without government-mandated surveillance would render ineffective.

## CONCLUSION

The situation of Synthetic Biology today is similar to the situation of genetic engineering 30 years ago. People expressed fear over bacteria producing substances that they do not produce naturally. For them, it spelled danger. But, today Genetic Engineering has helped in producing insulin in large quantities saving the lives of millions of diabetics.

Synthetic Biology also holds a similar promise. But to realize this promise, it needs active participation from researchers all over the world. Competitions such as iGEM are doing an excellent job in promoting this field among young scientists. But more academic, industrial and governmental participation is needed. Recently The University of California at Berkeley, the Institute for OneWorld Health, and Albany, California based upstart Amyris Biotechnologies received a grant of $42.5 million from the Bill and Melinda Gates Foundation for producing the antimalarial drug artemisinin in the lab with genetically engineered microbes [13].

The field today needs to build a strong foundation. We don't have precise tools and techniques in Synthetic Biology. The popularity of Synthetic Biology is likely to grow with the growing database of genome sequences. Further technology developments should be encouraged and supported. Commercial interests will drive more efficient and accurate synthesis of starting DNA sequences.

Along with the efficiency, the cost of DNA synthesis has reduced drastically from $10/bp in 2000 to under $1.5/bp today. It means that the cost of DNA synthesis has been cutting to half every 12–15 months. If this trend continues and is likely to continue, then the DNA synthesis can be made very affordable and viable for mass applications.

The fall in prices of DNA synthesis may also eliminate the problems arising in biological systems due to evolution. We may then create disposable genetic systems, which will counter the dangers, arising by the possibility of a mutation changing the biological system into a dangerous entity.

As far as tackling evolution is concerned, we should take note of how Viruses and other life forms cope with mutations. These life forms continue to perform more or less the same function even after mutations. So, new and radical approaches are needed to counter undesired evolution of the genetic circuits.

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