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An Overview on Synthetic Biology: its Classification, Engineering Approaches, and Applications of Synthetic Biology

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ABSTRACT

Synthetic biology is an interdisciplinary field that focuses on living organisms and systems, employing engineering techniques to create innovative biological devices, systems, and components. It represents the convergence of old and new approaches, bridging the gap between chemistry and biology, with synthetic chemistry laying the foundation for its emergence. At its core, synthetic biology aims to develop and engineer biological systems by bringing together engineers and biologists to design and construct novel biomolecular parts, circuits, and pathways. These constructs are then utilized to reconstruct, reanalyze, and reprogram organisms for various purposes. There are five primary categories within synthetic biology: bioengineering, synthetic genome, protocell synthetic biology, unconventional molecular biology, and in silico techniques. Traditionally, four engineering approaches have been employed in synthetic biology, including top-down, parallel, orthogonal, and bottom-up methods. These approaches provide a systematic and rational way of reassembling and reconstructing biological components, enabling the creation of functional biological devices, systems, and organisms with known, useful, and novel functions. Synthetic biology holds the promise of providing efficient solutions to various significant challenges in the modern world, encompassing areas such as chemicals, pharmaceuticals, agriculture, energy, and bioremediation. By leveraging engineering methods in the realm of biology, synthetic biology benefits from over 50 years of molecular biological and functional genomic research, along with advanced technologies that allow for the analysis, synthesis, assembly, modification, and transfer of genetic components into living organisms. In essence, synthetic biology offers an exciting avenue to unlock the potential of biological systems and revolutionize multiple industries through innovative modifications and breakthrough innovations.

Keywords: Synthetic biology; Artificial life; Biological systems; Bioengineering.

1. Introduction

Synthetic biology is a collaborative field of science that concentrates on living organisms and systems, and it uses engineering methods to develop new biological devices, systems, and parts [1]. The roots of synthetic biology can be seen in landmark research by Francois Jacob and Jacques Monod in 1961, in their research of *lac* operon in *E. coli* gave them to predict the presence of regulatory network that support the response of a cell to its surrounding [2]. In mid nineteenth century, the development of a detailed new direction in chemistry, instead of studying old molecules chemist began to synthesize them or making new molecules that did not exist before. This old and new approaches changed chemistry, this synthetic chemistry laid down the development of synthetic biology, an emerging field in which the goal is to develop biological systems [3].

The increase in molecular biology generated the field of synthetic biology, as computer scientists and biologists began to combine computation and experimentation to reverse engineer cellular pathways [4]. The study on restriction nucleases not only allowed us to make recombinant DNA molecules or to analyze single gene, but also took us into new world of synthetic biology where new gene can be constructed and analyzed [5]. In 2010, 1.08Mbps genome of *Mycoplasma mycoides* designed, synthesized, and assembled starting from digitizing genome sequence information and its transplantation into a *M. capricolum* (receiving cell) to create new *M. mycoides* cells that are controlled by the synthetic chromosome [6].

Synthetic biology is fetching engineers and biologists together to design and construct novel biomolecular parts, circuits, and pathways, and use these constructs to reconstruct, reanalyze and reprogram organisms [7]. Synthetic

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biologists divided into 2 broad classes, one uses the artificial molecules to reproduce emergent behaviors from nature biology, with aim of creating artificial life, and the other finds exchangeable components from natural biology to assemble into systems that function unnaturally [8]. Synthetic biology has a large scope, still in that it tries to recreate in unnatural chemical systems the evolving characteristics of living systems, like inheritance, genetics, and evolution [9].

Synthetic biologists search to assemble parts that are not natural to generate chemical systems that support Darwinian theory by carrying out the assembly in a synthetic way, these scientists hope to interpret non synthetic biology, that is natural biology [8]. Recently, an engineering team has given further. Meaning to the term. This community searches to extract from living systems exchangeable components that can be tested, validated as construction units, and reassembled to create devices that might or might not have counterparts in living systems [10] Synthetic biology already has many accomplishments to its credit. The effort to generate synthetic genetic systems has yielded diagnostic tools, such as Bayer's branched DNA assay, which annually helps improve the care of some 400,000 patients infected with HIV and hepatitis viruses [11].

2. Classifications of Synthetic Biology

There are five categories of synthetic biology that are bioengineering, synthetic genome, protocell synthetic biology, unconventional molecular biology, and *in silico* techniques [12].

(a) Bioengineering

The field of bioengineering focuses on making novel regulatory and metabolic pathways and these days drawing the attention of many researchers and funding. When using the word bioengineering it cannot be confused with traditional genome engineering, which involves adding a single gene into the organism. Bioengineer utilized synthetic biology to give more detailed and substantial ideas to alter organisms and biological systems. Bioengineering strives to achieve innovative biological systems by employing simplified and abstracted metabolic and regulatory modules, along with standardized parts that allow for flexible combinations to develop new pathways or organisms. This approach not only opens the door to countless novel applications but also promises greater predictability and control compared to conventional biotechnology [12].

(b) Synthetic Genomic

The development of animals with a chemically manufactured genome is another milestone of synthetic biology that is pointed out by synthetic genomics. The field synthetic biology contributed in the DNA synthesis technology, which now makes it easy to make DNA molecules with thousands of base pairs at minimal cost. The aim is to merge these molecules and transfer them in living organism cell, replacing the host genome or its metabolic pathways for different functions [12].

(c) Protocell Synthetic Biology

The generation of synthetic cells *in vitro* is the protocell branch of synthetic biology. Lipid vesicles can be used to create these cells because they have all the necessary function as a complete system. In the end, the aim is that these synthetic cells should be alive means capacity to self-replicate, self-maintenance, and evolution. Lipid vesicles can carry out specific functions with cell extracts and biological macromolecules. They may perform tasks like PCR or protein synthesis. Protocells offer versatile applications in synthetic biology, including biopolymer and medicine production [12].

(d) Unconventional Molecular Biology

The aim of unnatural molecular biology is to create new varieties of life that are based on different type of molecular biology, such as new types of nucleic acids and new genetic code. Novel nucleotides with distinct properties can be synthesized by modifying specific DNA or RNA components, such as the bases or backbone sugars. Organisms with genomes based on synthetic nucleic acids or new coding systems for synthetic amino acids could form a new type of life. This offers potential benefits but also introduces new risks. These synthetic organisms may lack the ability to exchange genes with natural species and could struggle to survive outside controlled environments if they escape. However, if they adapt successfully, they might possess advantages over natural organisms, such as resistance to predators and viruses, leading to uncontrolled proliferation [12].

(e) In Silico Technique

Different methods and synthetic biology in silico are connected to each other. The production of complex designs like metabolic pathways, vital cellular processes, whole genome, is one of the major obstacles faced by the methods mentioned above. That is why, synthetic biology has a strong in silico branch like system biology, that aims to make computational models for the design of common biological components or synthetic circuits for the stimulation of synthetic organisms [12]. Integrating the five domains of synthetic biology as a cohesive field of study is a logical approach. Despite their distinct focuses on different aspects of life, including metabolic regulation, essential elements, and biochemical makeup, these five strategies share a common goal: the creation of novel living organisms. Furthermore, the diverse methodologies employed in this field stem from various methodological approaches, contributing to the wide range of synthetic biology approaches [12].

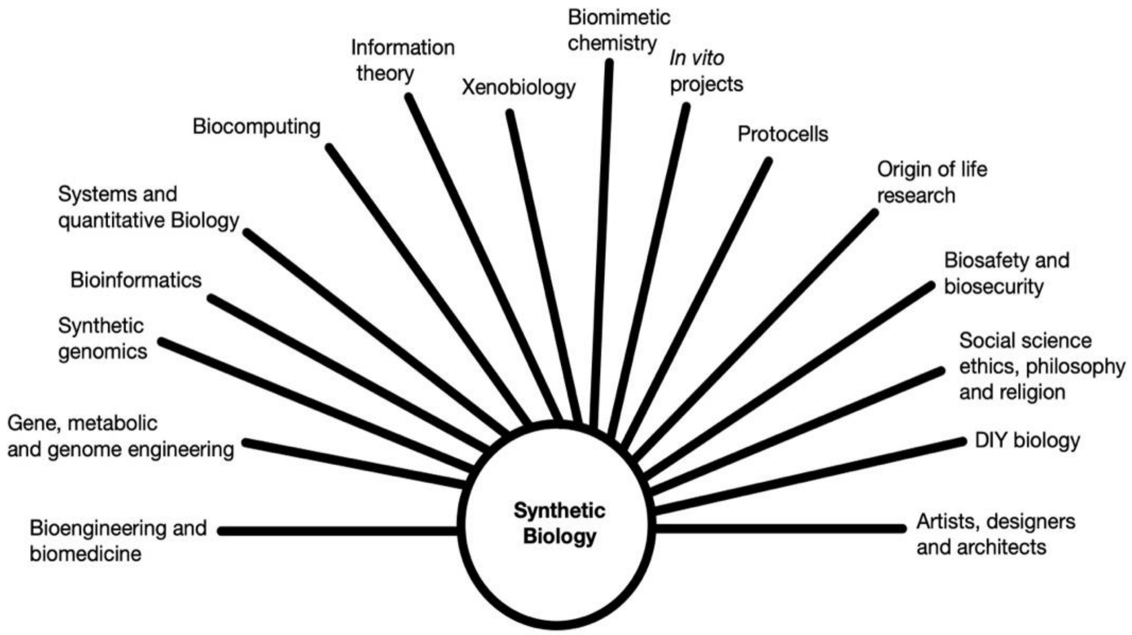

Figure 1. Overview of Synthetic Biology [13]

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3. Approaches that are Used by Synthetic Biology

Traditionally there were 4 engineering approaches that has been used by synthetic biology: top down, parallel, orthogonal, and bottom up [13].

Synthetic biology employs unnatural chemicals to replicate emergent behaviors found in natural biology, while also seeking to assemble interchangeable components from biological systems to create non-natural systems. This ambitious goal compels researchers to explore new frontiers and tackle challenges beyond the scope of conventional analysis. As a result, novel paradigms emerge, driving innovation in ways that traditional analysis alone cannot achieve. Beyond creating playful gadgets like oscillating or creeping devices, synthetic biology has also given rise to diagnostic instruments that significantly improve the treatment of patients with infectious diseases [8].

(a) Top-Down Approach

Synthetic biology utilizes metabolic and genetic engineering techniques to introduce new functionalities into living cells (Schwille, 2011). One approach involves comparing universal genes and removing non-essential ones to create a simplified genome, aiming to reduce the complexity of existing cells. However, recent research suggests that the tree of life, composed of eukaryotic and prokaryotic cells, may have evolved from a group of primordial cells rather than a single cell. Consequently, the quest for the "minimum genome" akin to the Holy Grail has become elusive, as eliminating non-essential functions can compromise an organism's fitness and result in "fragile" genomes [13].

(b) Bottom-up Approach

This approach involves the creation of new biological systems in vitro by assembling 'non-living' biomolecular components,[15] often with the goal of constructing an artificial cell. The three fundamental self-organizational principles considered for this purpose are reproduction, replication, and assembly. Reproduction involves the concept of "hardware" in cells, where a container and metabolism are combined. Replication, on the other hand, pertains to systems duplicating perfect copies of themselves, like DNA, which is akin to "software." Assembling occurs when vesicles or containers, such as Oparin's coacervates, formed of tiny droplets of organic molecules like lipids or liposomes, aggregate to create membrane-like structures composed of phospholipids [13].

This area of study, including protocells and other in vitro synthetic biology initiatives, aims to produce minimal cells, metabolic pathways, "never-born proteins," and mimic physiological functions like cell division and growth. Although the in vitro enhancement of synthetic pathways may no longer be strictly classified as synthetic biology research, it holds significant potential to impact other synthetic biology sectors, including metabolic engineering. This essential research warrants proper recognition as a part of synthetic biology exploration [13].

(c) Parallel Approach

Parallel engineering, also known as bioengineering, is rooted in the fundamental genetic code, utilizing conventional biomolecules such as nucleic acids and the 20 amino acids to construct biological systems. This versatile field encompasses various applications in biocomputing, bioenergy, biofuels, bioremediation,

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optogenetics, and medicine. It involves standardizing DNA components, engineering switches, biosensors, genetic circuits, logic gates, and cellular communication operators. To direct the expression of two or more genes and/or proteins, many of these applications rely on the use of vectors or plasmids. Plasmids are small, circular, double-stranded DNA units commonly found in prokaryotic cells, and occasionally detected in eukaryotic cells, capable of autonomous replication independent of chromosomal DNA [13].

(d) Orthogonal Approach

Also known as perpendicular engineering or chemical synthetic biology, this approach aims to modify or expand the genetic codes of living systems using artificial DNA bases and/or amino acids. Connected to xenobiology, this field explores the use of "alien" or xeno molecules as genetic information carriers by creating compounds like DNA canonical bases [13]. By altering or enlarging the genetic code, information beyond the 20 conventional amino acids can be expressed. Researchers have successfully incorporated noncanonical moieties into DNA and proteins, leading to advancements in creating biological systems with diverse biomolecules. Directed evolution is utilized to produce orthogonal enzymes, enabling the incorporation of XAAs into proteins and the development of "mirror life" systems [13].

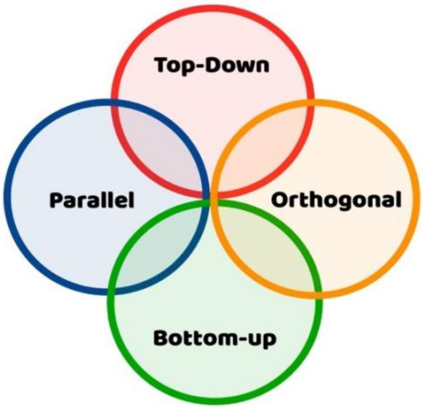

Figure 2. Proposition of four engineering approaches encompassing all synthetic biology research [13]

4. Applications of Synthetic Biology

Synthetic biology guarantees to give efficient solutions to many big problems of modern world through modifications and innovations in chemicals, pharmaceuticals, agriculture, energy, and bioremediation [16]. By using engineering methods to biology, synthetic biology has become the science of reassembling and reconstructing biological components in a systematic and rational manner to create and engineer functional biological devices, systems, and organisms with known, useful, and novel functions. Synthetic biology has ability to use different biomolecular parts compiled over 50 years of molecular biological and functional genomic research [17] as well as technology that has made it possible to analyze, synthesize, assemble, modify, and transfer genetic components into living organisms [18].

(a) Biosensors

A biosensor is an engineered organism, often a bacterium, designed to detect and report specific environmental conditions, such as the presence of heavy metals or toxins. One notable example is the Lux operon found in

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Aliivibrio fischeri, [19] which encodes for the enzyme responsible for bacterial bioluminescence. By placing this operon downstream of a responsive promoter, the luminescence genes can be expressed in response to a particular environmental stimulus [20]. One application of this technology involved creating a biosensor with a bioluminescent bacterial coating on a photosensitive computer chip to detect petroleum pollutants. When the bacteria detect the pollutants, they emit luminescent light, providing a visible indication of the presence of contaminants. Another instance is the use of an engineered E. coli reporter strain capable of detecting TNT and its main degradation product DNT, resulting in the production of green fluorescent protein (GFP) when these substances are present. This mechanism allows for the detection of landmines using the bacteria as a sensing tool. These biosensors offer promising opportunities for environmental monitoring and detection of various substances in a reliable and cost-effective manner [21].

(b) Biological Computers

A biological computer is a product of engineered biological systems capable of performing computer-like operations, representing a significant paradigm in synthetic biology. Scientists have successfully constructed and analyzed various logic gates within different organisms, [22] showcasing both analog and digital computation abilities in living cells. Through their research, they have shown that it is feasible to engineer bacteria to execute analog and/or digital computations, opening up new possibilities for advanced biological computing [23].

(c) Cell Transformation

Cells use gene circuits, composed of interacting genes and proteins, to carry out various functions like responding to signals and making decisions. Synthetic biologists have designed gene circuits that control gene expression at different levels, such as transcriptional, post-transcriptional, and translational levels. With the introduction of foreign genes and directed evolution, traditional metabolic engineering has been advanced, enabling the production of valuable substances like the precursor of the antimalarial drug, Artemisinin, [24] in E. coli and yeast. Although creating entire organisms from scratch is still a challenge, new DNA can be integrated into living cells to bestow them with desired capabilities and phenotypes. Through cell transformation, biological circuits can be constructed and manipulated to achieve specific outputs [25].

(d) Designed Proteins

Natural proteins can undergo engineering through techniques like directed evolution, leading to the creation of novel protein structures that can match or even surpass the functionalities of existing proteins. For instance, one group developed a helix bundle that mimicked hemoglobin's oxygen-binding properties but didn't bind carbon monoxide [26]. Another group produced a structurally and functionally novel ATPase [27], while a different protein structure supported various oxidoreductase activities [28]. Computational approaches are also employed to engineer novel functionalities or protein specificity. In one study, two computational methods were utilized: a bioinformatics and molecular modeling method to mine sequence databases, and a computational enzyme design method to reprogram enzyme specificity. These methods resulted in designed enzymes with significantly enhanced specificity for producing longer chain alcohols from sugar [29]. Furthermore, researchers often explore expanding the natural set of 20 amino acids. While 61 codons have been identified, generally only 20 amino acids are coded in

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all organisms. By engineering certain codons, alternative amino acids like O-methyl tyrosine or exogenous amino acids such as 4-fluorophenylalanine can be incorporated. These projects often rely on re-coded nonsense suppressor tRNA-Aminoacyl tRNA synthetase pairs from other organisms, requiring substantial engineering in most cases [30].

(e) Designed Nucleic Acids

Scientists have achieved the remarkable feat of encoding digital information onto a single strand of synthetic DNA. Back in 2012, George M. Church successfully stored one of his books on synthetic biology using DNA, totaling 5.3 Mb of data, which was over 1000 times larger than the previous record for information stored in synthesized DNA [31]. Another exciting project involved encoding William Shakespeare's complete sonnets in DNA. In the broader context, several algorithms like NUPACK, ViennaRNA, Ribosome Binding Site Calculator, Cello, and Non-Repetitive Parts Calculator [32] have been developed, enabling the design of novel genetic systems. Furthermore, various technologies have emerged for incorporating unnatural nucleotides and amino acids into nucleic acids and proteins, both in laboratory settings (in vitro) and within living organisms (in vivo). These advances represent significant milestones in the field of synthetic biology [33].

- Utilizing microorganisms for bioremediation to remove contaminants from water, soil, and air.
- Modified rice produces beta-carotene, a substance preventing vitamin A deficiency, benefiting hundreds of thousands of children at risk of losing their vision and dying from infectious infections.
- Yeast is engineered to produce rose oil, offering a sustainable and environmentally friendly alternative to using fresh roses for perfumery.

Some other applications are space exploration, synthetic life, drug delivery platforms, engineered bacteria, engineered yeast, cell-based platforms, biofuels, pharmaceuticals, and biomaterials.

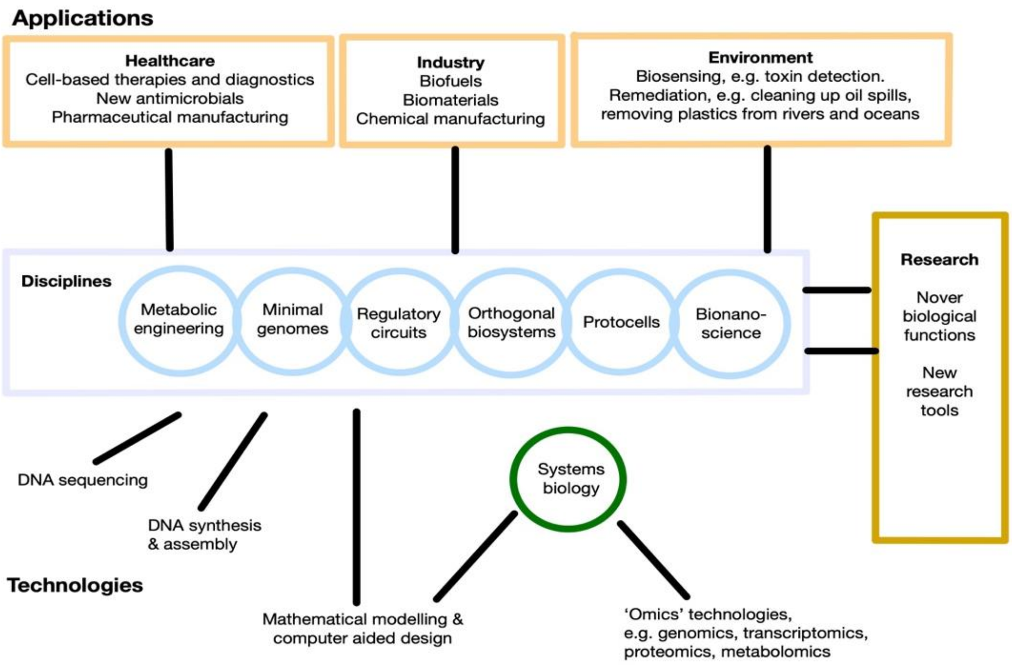

Figure 3. Overview of Synthetic Biology [34]

Declarations

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Conflict of Interest

The authors declare that they have no conflict of interest.

Consent for Publication

The authors declare that they consented to the publication of this study.

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