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Perspective Article

Defining and Redefining its Role in Biology: Synthetic Biology as an Emerging Field at the Interface of Engineering and Biology

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Abstract

Synthetic biology is often misunderstood as creation of artificial life or new biology using principles different from those of extant organisms around us. But, fundamentally, the field is about engineering biology in a more efficient and effective way and endowing new functions in existing organisms using a more refined and predictable approach. Thus, synthetic biology as encapsulated by the field it helps defined is enhanced recombinant DNA technology, an example of which is modular and orthogonal "standard swappable biological parts". But, as the field grows and matures, various "allied" fields are subsumed into it such as metabolic engineering, protein engineering, directed evolution, origins of life research, and systems biology, which in totality represents a new perspective of how engineering principles can be utilized to expand, in scope and depth, the realms of questions that biology ask. Two parallel approaches, directed evolution and de novo protein design, are frequently used to engineer new phenotypes into organisms. Similar to evolution but with purposeful use of selection pressure to elicit progressive refinement of specific traits in an efficient manner, directed evolution is a powerful methodology that generates, at the cell level, libraries of mutants of slightly different function such as differing resistance to heavy metals, that upon exertion of continued selection pressure, led to the evolution of a strain capable of thriving under a hostile environment previously inhabitable to the organism. Taking a different approach, de

novo protein design taps on advances in biomolecule structure modelling together with bioinformatic sequence search for inserting, in a structure defined manner, specific amino acids (natural or unnatural) in a protein structure to endow desired functionality, where one highly sought function is catalysis of unnatural reactions such as the Diels-Alder reaction. Long chain length DNA synthesis, on the other hand, finds utility in enabling the synthesis of a minimal genome for a bacterium, which demonstrates the huge possibilities of having a microbe with an optimized genome (free of extraneous genes) for biotechnological applications in delivering drugs and fuel at high titer with lower cost. Having assimilated other fields, synthetic biology is again redefining its role as its seeks to use, in an ethical and responsible manner, a new way of adding new functions into organisms through genome editing. For example, CRISPR/Cas9 genome editing holds enormous potential for providing lifesaving gene editing capability in medical treatments, while enabling fast, easy removal of undesirable genes and prophages from a production microorganism. Synthetic biologists are asking themselves deep questions on how best to regulate this powerful technology that could be as impactful on science and human society as recombinant DNA technology was in 1973.

Keywords: DNA synthesis; De novo protein design; Directed evolution; Modular genetic parts; Metabolic engineering; Minimal cells; Metabolic flux analysis; Protein engineering; Recombinant DNA technology; Systems biology

Subject Areas: Bioengineering; Biochemistry; Chemical engineering; Cell biology; Molecular biology

1. Perspective

When it was first demonstrated in 1973, genetic engineering or recombinant DNA technology was much worried as it holds potential for altering the genetic makeup of organisms, and thus, may be the trigger for infectious disease spread or release of dangerous microorganisms from labs that research on genetic engineering. Many decades on, the fear has subsided as the public gradually understood the technology and the many positive benefits it brings. Dangers of microbes picking up new genetic repertoire that may endanger the ecosystem are largely in check due to natural constraints on the transmissibility of non-essential traits. Fast forward to the early 2000s, a new research area of synthetic biology gradually coalesces into an emerging field still in development and definition. Specifically, the desire to tinker with genes on a larger scale compared to the one gene at a time approach in established recombinant DNA technology led to ideas of bringing an engineering approach to biological research. For example, synthetic biologists aim to alter specific genes in the genome with high specificity and fidelity; thereby, enabling desired traits to be conferred to particular microorganisms with greater ease and speed. More importantly, the conventional approach to genetic engineering is laborious and time-consuming, where multiple iterations of adjusting gene dosage and type of promoters are necessary for heterologous DNA to be expressed in a recombinant organism. Trying to do much better than the status quo, synthetic biologists aim to create "standard biological parts" comprising modular genetic units that could be easily transferred into microorganisms for the expression of new proteins. Going beyond single genes, the longer-term goal would be the facile transfer and integration of entire segments of genes into a host organism for the reconstitution of a missing metabolic or signalling pathway

or expression of new functions. But, synthetic biology is larger than genetic engineering, its most popular definition describes its role as a broad field that aims to use enhanced tools of genetic engineering and high throughput screening for conferring new and beneficial functions to organisms. Articulation of this goal would naturally mean that desired mutations must be reliably engineered into the genome. Thus, comes the enabling technology of DNA synthesis that facilitates the design of specific nucleotide sequences with point mutations precisely inserted for understanding the role of specific changes in DNA on protein function. This is a step change from the use of error prone Polymerase Chain Reaction (error prone PCR) for random insertion of mutations in a specific stretch of DNA in terms of specificity, speed, and reliability, and with the help of DNA synthesis companies synthesizing specific DNA fragments on order, greatly accelerates genetic engineering research. With the ability to expand the sequence search space for new functions comes the problem of identifying the phenotype of interest, to which high throughput screening approaches helps to address. Specifically, fluorescence tagged molecules expressed when certain conditions are met in the cell or more traditional biochemical assays can be used for screening desired traits conferred by sought after mutations that, for example, enable the formation of novel types of chemical bonds such as that between silicon and carbon [1]. Enlarged to a larger scale where thousands of samples can be sampled and analysed by robotic means, high throughput screening technology searches the sequence space generated by random mutagenesis that, hopefully, would yield the desired function.

Random generation of mutations by chemical means, however, may not be sufficient for pointing the development of a sequence of mutations that change the function of a protein. To this end, the approach of directed evolution [2] find use in exerting selection pressure on a microbial species for evolving successively better solutions to an environmental stressor or existential threat. Usually, a chance occurrence amidst a sea of thousands or millions of mutations requiring examination, high throughput screening find ready use for automating and reducing the search time for profiling the desired trait. The key in facilitating the search for the target mutation is in developing a simple yes or no response that either could be colour-coded or encapsulated in a growth response. Like cells that grow giving a "yes" answer, a high throughput screen based on a similar concept would enable a rapid targeted search of a vast genetic space. Desire to engineer biology for beneficial uses such as producing fuel molecules from sunlight capture meant that multiple traits must coexist in a cell to enable the complex sequence of chemical transformation to occur sequentially in situ for the desired outcome to manifest at the population level. But how do we fathom about the complexity of biological networks that interconnect pathways using conceptual tools that usually apply to single enzyme cascade. Enter the conceptual engine of systems biology that seeks to understand, holistically, the complicated interplay between proteins and genes as well as metabolic flows in channeling biological building blocks into energy powering movement, biological computing and chemical decision making. Beyond understanding, the mathematical tools developed for systems biology to glean aggregate information from molecular processes could also be used, in reverse, to develop strategies for facilitating enhanced production of specific metabolic precursors for final drug production in a microbe. Known broadly as metabolic engineering [3, 4] where metabolic flux analysis identifies the crucial nodes and proteins that are choke points of metabolism in a native host or recombinant

organism, modulation of gene dosage or promoter strength are common tools for channelling metabolite flux through one pathway over another that, phenotypically, translates into production of a fuel molecule or enzymatically decorated glycosidic drug molecule functional in treating human diseases. Moving forward, genome editing [5, 6] provides an alternative view of genetic engineering different from that of the additive recombinant DNA technology where new genes (and usually functions) are added to organisms. Specifically, genome editing seeks to identify and reproducibly alter a specific gene for gaining or losing a function [7] For example, the high specificity and fidelity tool of CRISPR/Cas9 (clustered regularly interspersed short palindromic repeats/Cas 9) approach is able, with the help of a guide RNA, locate a specific stretch of target DNA (complementary to the guide RNA sequence) and perform molecular cleavage with few nucleotide resolution [8]. Doing so, a defective gene could be removed or inactivated, while combining with other approaches, a new gene could be introduced [9] Useful for en masse removal of prophages from a genome, the technique has also been used for editing specific non desirable traits in embryos, though the latter use raise serious ethical considerations. Thus, one and a half decade on, the field of synthetic biology remains emerging and is continuously defining its role in biological research. Trusted with the objective of making genetic engineering faster and with higher fidelity and able to orthogonally introduce new traits into established hosts without upsetting cellular processes, standard biological parts represent a first foray into making biology engineer able like an electronic circuit with swappable parts. On the other hand, new traits are left to evolve on its own in cells put through successive rounds of selection pressure in an approach known as directed evolution. Observed through high throughput screens able to quickly search a large library of mutations covering a big sequence space, directed evolution allows mutations to be selected by natural selection to survive under specific external pressure. This is diametrically different from the de novo protein design approach where specific mutations are designed into a sequence for conferring a desired point mutation [10-12]. But, both approaches are useful for introducing specific mutated genes into organisms that could help transform a cellular metabolite into desired compounds after the metabolic circuitry has been examined under metabolic flux analysis and other system biology approaches; thus, filling a gap in natural catalytic wizardry. The future holds real promise on the use of DNA synthesis technology for the creation of large genomes for examining the genetic and biological basis of minimal cells in understanding the origins of life, or on a more practical note, improving the efficiency and effectiveness of drug and fuel production through processes encapsulated in a minimal but optimized microbial genome. However, advent of genome editing, while promising for reproducible alteration of large numbers of genes sprouting the same genetic identification tag as a guide RNA, is also fraught with dangers of misuse for designing embryos with target traits.

Conflicts of Interest

The author declares no conflicts of interest.

Author's Contribution

The author wrote the manuscript.

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