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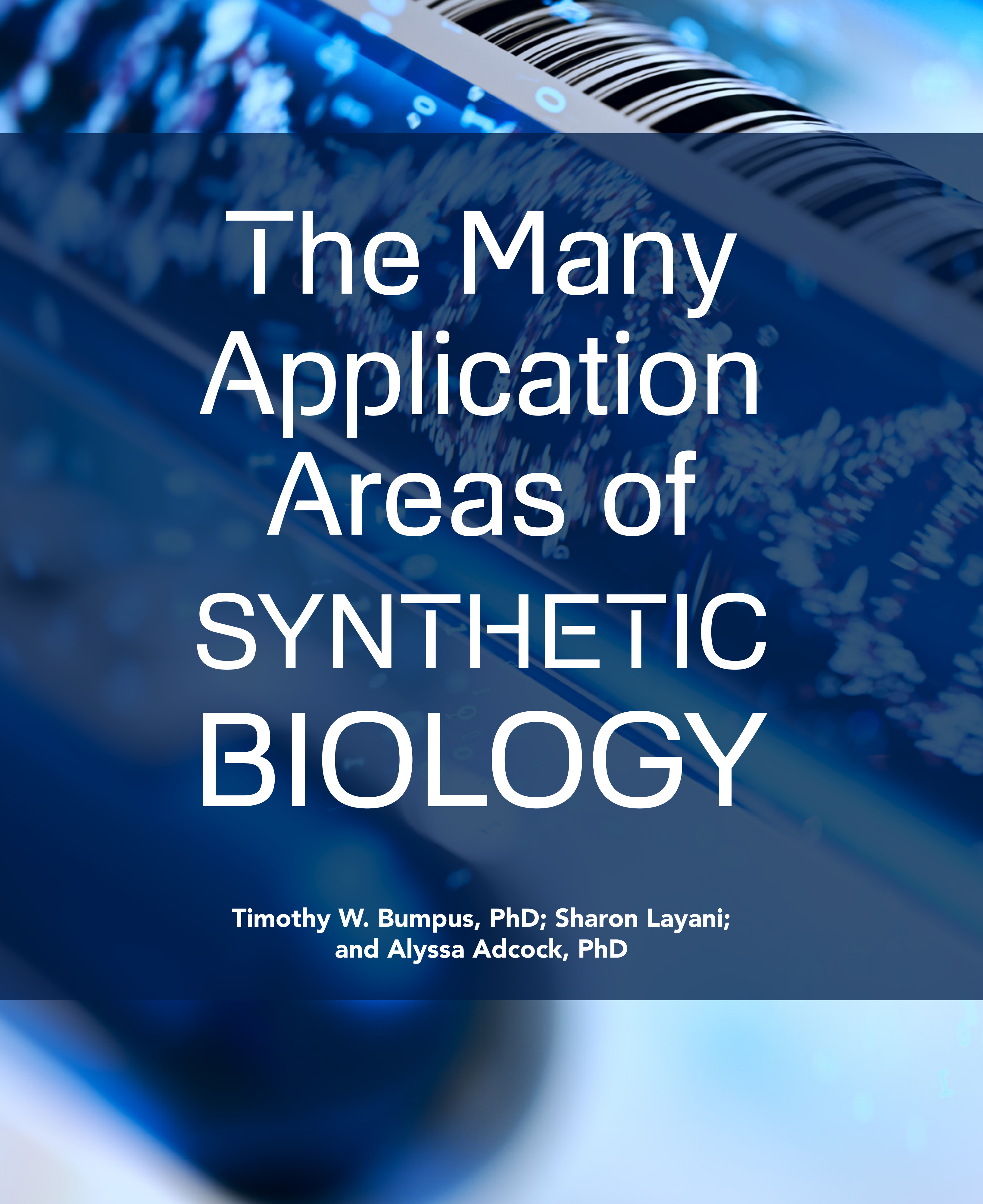

The Many Application Areas of SYNTHETIC BIOLOGY

Timothy W. Bumpus, PhD; Sharon Layani; and Alyssa Adcock, PhD

The burgeoning field of synthetic biology promises myriad applications that would mark a major shift in the lives of people worldwide. In the same way that microelectronics transformed society beginning in the latter half of the 20th century, synthetic biology and its applications are likely to again transform society's relationship with products and nature. Such profound change arises because through synthetic biology, humankind can reach beyond exploiting nature and its biological factories as developed through eons of evolution and begin designing biological products with structures and properties dictated entirely by us.

The advertised applications of these new technologies include developing new classes of therapies and vaccines (which we have begun to see with the mRNA COVID-19 vaccines), the ability to cure previously intractable diseases, or the possibility of eliminating genetic diseases. Much focus has been placed on the possibility of editing human embryos (so-called germline editing), which could result in inheritable changes to the human DNA pool, and the consequent dangers. However, there are other applications that do not require heritable modifications (somatic cell editing), and still others that do not modify the human genome at all. Our interest here is in the applications outside of human germline editing. Further, there are important applications beyond medical interventions, to include environmental care, agriculture and food production, materials production and manufacturing, energy management, and (even) information technology. With so many diverse applications, it is useful to discuss some of the possibilities in each area.

Introduction

The term "synthetic biology" was coined over a century ago. Since then, synthetic biology has grown into a diverse, multidisciplinary field that leverages tools, techniques, and ideas from biology, chemistry, engineering, computer science, medicine, bioinformatics, and many other fields. Closely allied to bioengineering, synthetic biology aims to create new biological elements or redesign existing processes found in nature. Recent scientific breakthroughs and commercial tools have ushered in new advancements and opportunities. The development of these tools and the maturation of synthetic biology as an academic field has led to the discovery of applications and the formation of companies.

The current field of synthetic biology is the culmination of decades of scientific breakthroughs and technological advancement. From the discovery of DNA and its function, to uncovering its double-helix structure, unraveling the genetic code, constructing a reference human genome, economizing genetic sequencing, and the more recent developments in genome editing, these basic research discoveries have enabled innumerable applications. In his recent book, author Walter Isaacson describes the CRISPR-Cas-9[1] "genetic scissors" discovered by 2020 Nobelists Jennifer Doudna and Emmanuelle Charpentier as transforming "the future of the human race"[2] and flags the many thorny ethical issues this transformative technology brings to the fore. Most notable of these are the issues surrounding germline editing, which would alter the inheritable human genome.[3] However, these concerns, though extremely serious and justified, should not forestall the use of synthetic biology in its many other applications.

Depending on what one includes in the breadth of synthetic biology, market potentials for applications (in annual US dollar amounts) are estimated to be in the tens of billions for new applications over the next few years.[4] Venture capital funds [5] and foundations[6] have been established to support and accelerate developments. Dozens of early-stage start-up companies have been formed in the US, while at the same time basic research continues in academic and corporate research laboratories to provide greater understanding of the opportunities afforded by synthetic biology. Bringing applications to fruition and commercializing synthetic biology products, however, will require considerable work and technological expertise.

It is clear that the field is in its infancy, and thus the full range of applications remains largely unexplored. Many applications, as yet unimagined, might be possible as the field expands. For example, it is possible to modify DNA to admit replacements for one or more of the four nucleotides, or to expand the number and kind of nucleotides.[7] One such experiment used eight different nucleotides.[8] Such an expanded genetic alphabet will surely allow researchers to explore new possibilities and may result in new proteins and polynucleotides with previously unattainable, or even unimaginable, properties and functionalities. While some researchers work to build new biological systems and capabilities from the ground up, others are starting with the nearest facsimile nature already possesses and then through directed evolution teaching that protein new, alternative functions. This allows for biology's catalysts (enzymes) to do more of the work that was once done by teams of human chemists.

In this article, we discuss the current activities in and progress across a number of synthetic biology application areas. In all cases, however, much more development can be expected—leading to a range of new products, many not envisioned here. As the academic field continues to organize and expand, the range of applications for exploration will likewise expand, through startups and industries. Our focus here is on what is known now about applications and their logical extensions.

Human Health Care

In 2020, we learned how messenger RNA (mRNA) can be designed to instruct human cells to produce a protein based on a portion of the SARS-CoV-2 virus that causes COVID-19, thereby teaching the immune system to produce antibodies (the body's major element of immunological defense) prior to an infection. The rapid development of this novel vaccine has profoundly impacted society and promises future vaccines and therapies based on an analogous approach. Based on rapid genetic sequencing, computational biology, and biomanufacturing, the Moderna and BioNTech/Pfizer vaccines have become advertisements for the success of synthetic biology.

The production of mRNA vaccines is but one of the successes of synthetic biology in biomedicine. Engineered yeast have been used to produce semi-synthetic artemisinin for an anti-malarial drug,[9] and production of the diabetes drug Januvia® uses a new directed evolution derived enzyme to replace older, environmentally harmful manufacturing processes.[10] A new form of cancer treatment called CAR-T cell therapy[11] (for Chimeric Antigen Receptor T-cell therapy) uses synthetic biology to alter T-cells harvested from the patient's body to attack tumors. The success of CAR-T cells has sparked interest in applying the same technique to other immune cell types, such as natural killer cells.[12] Other interventions under development use engineered probiotics to augment the body's immune functions through the gut's reservoir of commensal bacteria.[13] Yet other developments use synthetic biology to generate 3D scaffolds upon which to culture cells and regenerate bones, tissues, or potentially even whole organs.[14]

Successes to date just scratch the surface of possibilities for synthetic biology in the design of novel diagnostics, therapeutics, vaccines, and preventative health care. Future applications include further vaccine development (potentially again using mRNA vaccines) to thwart a host of viral afflictions (for example, the common cold), prophylactic healthcare, treatments for metabolic disorders, and stopping emerging infectious diseases *before* they become pandemics. Further, it can be applied to uncover biologics that counter the effects of aging on the human body.[15]

Because market forces will drive demand, we can envision that synthetic biology will also serve the interests of performance enhancing drugs, supplements, and production of unapproved pharmaceuticals. In these cases, efficacy is likely to be a mixed bag as it will be based on understanding the biological mechanisms of the human body, which are immensely complex. Synthetic biology will be a component of the research that increases our understanding, but it is not guaranteed that all biologics will provide safe and effective results, regardless of the method of production.

Going forward, we can expect synthetic biology to be the crucial enabler for precision medicine.[16] Precision healthcare, which includes individualized medicine, is often regarded as the future of human healthcare. Precision health would revolutionize the practice of medicine by tailoring diagnostics and therapies to individuals based on their unique genetics, microbiome, diet, lifestyle, environment, or individual disease characteristics. Diagnostics and therapeutics may then be tailored based on those particulars, making use of synthetic biology where necessary and appropriate.

Earth's Biological Environment

The tools of synthetic biology can be applied by humans to augment, improve, and better exploit the earth's biological environment. That environment consists of the delicate ecosystems that surround us and with which humans must coexist. Over the millennia, humans have profoundly impacted the biological environment, often warring against pests and threats while exploiting other plants and animals for our benefit. Synthetic biology provides unique opportunities and capabilities to further these influences on the environment, ideally in beneficial ways.

Fighting undesirable, disease carrying, or invasive species may now be accomplished through synthetic biology, in preference to the introduction of competitor species.[17] For example, genetically modified mosquitoes are being developed to curtail the spread of mosquito-borne diseases, which remain a serious threat to human health.[18] Field trials have recently begun in the Florida Keys to test both the efficacy and effects of such approaches in natural ecosystems. A biopesticide approach to controlling locust infestations has also been proposed, using synthetic biology to reprogram the insects' microbiome and disrupt essential genes via bacteriaphages.[19] Gene drives (a genetic construct that alters the genetic makeup of a particular species by inserting a small piece of DNA into an individual that then spreads and dominates reproduction of genes in subsequent generations) and viral vector delivery systems are highly modular approaches that will likely find many new applications, to include addressing infestations, limiting the spread of zoonotic diseases, or culling invasive plants or animals.

Other applications of synthetic biology to altering the earth's biological environment can involve generating microbes with desirable properties.[20] Such developments might portend an ability to clean up oil spills or remediate other forms of environmental and xenobiotic pollution. Application of such microbes may hasten the degradation of plastics in landfills,[21] promote nitrogen fixation, improve soil productivity, and reduce fertilizer usage,[22] or even reduce atmospheric greenhouse gas levels.[23] Synthetic biology can also enable the development of biological markers that can be used as sensors, to detect the presence of contaminants or pollutants. One such research development involves

bacterial infused beads that can be spread across the surface of a field and which fluoresce green in the presence of trinitrotoluene (TNT) vapors to detect buried landmines.[24] Indigenous plants might also be modified in a similar way, so as to include sensors that can be observed optically or by other spectroscopic means.

In the realm of multicellular creatures, there have been serious thoughts about bringing back the wooly mammoth through synthetic biology,[25] based on genetic information found in preserved DNA strands. Such techniques could also be used to restore recently extinct species or re-establish species that are nearly extinct. This could include, for example, rescuing certain species of trees from a blight, or treating a species of beneficial bees to resist specific diseases.

Admittedly, many of these applications come with profound risk. Humans have often caused grave environment damage, frequently as a result of good intentions gone awry. Modern interventions using synthetic biology must be carefully tailored to the specific problem and have safeguards built in to prevent undesirable effects. These are challenging issues, spanning scientific disciplines and jurisdictional regimes. Special caution is warranted because many forms of synthetic biology involve unleashing life forms that replicate and proliferate.

Agriculture and Food Production

While controversial today, genetically modified organisms (GMOs) have been produced historically through animal husbandry and agricultural practices involving plant breeding. However, now, it is possible to apply synthetic biology approaches to add or subtract genes to plants and animals to produce desirable benefits. Viral transduction, which uses a virus to transfer genetic material, has been used to produce hardier plants.[26] Famously, Monsanto developed seeds (such as soybeans) that are immune to the deleterious effects of the weed killer Round Up® thereby allowing for greater crop yields.

Less notorious uses of synthetic biology for agricultural purposes are found in the development of fertilizers rich in engineered bacteria to improve crop yields,[27] and the development of artificial food additives using custom fermentation.[28] The latter products can include sugars, flavor simulants or enhancers, dietary supplements (such as flavonoids and terpenoids), and oils and fats (lipids) for food enhancement. Using knowledge of human taste and olfactory receptors, it might be possible to develop new flavors of foods more pleasing to human senses. Looming on the horizon, and of much interest in agriculture, is the use of synthetic biology to culture meat directly from engineered cells, obviating the need to butcher animals. Already, the FDA has approved a fish food that has been developed through the editing of a bacteria's genetic makeup that produces a flour-like aquaculture feed.[29] While seemingly far from growing a synthetic beef steak, numerous companies and research labs are in a race to develop cultured meat with properties identical to animal products.[30] Some predict that within five years, consumer cultured meat products will be commonplace.[31] Others believe that getting the texture, grain, and mix of fats to properly mimic actual butchered meat will prove more difficult. The technology to make it happen, however, includes a number of synthetic biology concepts, as well as 3D printing technology using multiple constituent cell types.

Similarly, other food products, to include fruits and vegetables, might be cultured from media using biological processes that do not rely on natural soil and sunlight. Efficiencies in food production using synthetic biology to culture cells, whether meat or vegetable, extend beyond the rate and aesthetics of production. Consider the situation of a contained bioenvironment, such as a long-duration manned space capsule, or an Antarctic outpost. Production of fresh food via cell/tissue culture, as opposed to long term storage or resupply using long transport lines, can offer major advantages. Further, by using recaptured water to reconstitute freeze-dried media, the total weight of the food supplies can be significantly reduced. Beef, for example, is around fifty percent water, so by culturing cells in reconstituted media to produce a juicy New York Strip, you could eliminate up to half the weight of the beef supply, while supplying a superior fresh food product.

Synthetic biology might also be able to detect and reduce issues of food spoilage. Biological sensing mechanisms embedded into the packaging may monitor the state of the food degradation, such as detecting milk spoilage.[32] Today, some foods are irradiated to prolong their lives, which is an indiscriminate way of adjusting the biological makeup. Additives or packaging designed and manufactured via synthetic biology would be able to more precisely counter processes that cause food spoilage, producing foods that require less in the way of refrigeration to achieve long shelf lives. Such biologic sensors and additives could dramatically transform food distribution and storage.

Materials Production and Manufacturing

Biological processes produce a large variety of proteins with diverse structural and chemical properties. From delicate silk thread fibers with immense tensile strength, to the cellulosic structures to enable trees to reach their towering heights, natural biology can construct and manufacture an amazing array of useful structures. Through synthetic biology, humans may also start to make use of biological processes to produce specially designed materials and construction methodologies. By replacing or modifying traditional processes such as chemical synthesis and commodity manufacturing, we are likely to design novel materials and new applications, all the while improving the efficiency and reducing the environmental impact of manufacturing.

Though biomanufacturing remains in development, both existing materials companies (for example Dupont) and innumerable start-ups are exploring how best to leverage biological processes to generate products. Bio-cements, for example, are being produced to create materials and adhesives for construction that reduce its carbon footprint.[33] There is also a long history of attempting to produce silk thread, normally associated with spiders and silkworms, which thanks to synthetic biology is beginning to achieve meaningful success.[34] Commercial-quality nylon 6 yarns, films, and engineered bioplastics are the intended products of a joint venture that produces bio-renewably derived caprolactam (a precursor to these products).[35] These replacement products are intended to be more sustainable and environmentally friendly. The Department of Defense has awarded a Manufacturing Innovation Institute, called BioMade, to the Engineering Biology Research Consortium led by Cargill to "identify and innovate on shared challenges in scaleup and downstream processing to further strengthen the US economy in the production of bioindustrial products."[36]

Researchers are using synthetic biology to develop cell-free protein synthesis methods. Proteins are typically produced in cells, where cellular membranes render these biochemical wunderkinds largely inaccessible. Synthetic biology now permits protein synthesis without cell membranes, to scale up production of therapeutic small molecules[37] and glycoproteins,[38] fine chemicals, biofuels, and even bio-enabled smart materials.[39] Cell-free synthesis brings to convergence materials science and biomanufacturing at the nanoscale.

Biology can also combine, stack, and recombine nanostructures to produce macrostructures, such as plants and animals. Functionally graded materials differ from bulk or composite materials in that mixtures and arrangements can be deliberately adjusted within the material. The chemistries and precise spatial control required to build structurally strong, lightweight materials, or materials with corrosion resistant surfaces or embedded sensors, are difficult processes with standard chemistry and manufacturing, but perhaps possible with biological manufacturing. Our mastery of synthetic biology is likely to be key to developing a large range of such materials.

Energy Production and Management

Fundamentally, biological organisms are simply energy management systems that take in energy in the form of food and/or sunlight, perform energy conversions, and then expend or store that energy. Commercial industry (a decidedly non-biological creature) is now looking to synthetic biology to mimic some of these processes to create artificial photosynthesis, create biofuels, and develop bio-battery energy storage capabilities. Materials that can help catalyze or facilitate ion transport are important to quality batteries, and synthetic biology might generate specialized materials for applications in this domain of battery refinement.

Current capabilities use engineered *E. coli*[40] to convert waste materials (such as wastepaper and carbohydrates) or microalgae[41] harvesting the sun's energy to produce diesel fuel alternatives. While much vaunted, such technologies have, to date, attained limited commercial success. Another project is researching the use of modified DNA to encode enzymatic processes that convert complex organic fuels (likely, waste products) into energy.[42] Researchers are also very interested in harvesting solar energy through a synthetic form of photosynthesis, thus converting the sun's plentiful photons to accessible chemical or electrical energy.[43]

Artificial lighting is a major consumer of electrical energy, but can potentially be replaced by bioluminescence, enhanced using synthetic biology. Far from being a jar of fireflies, engineered luminescent microorganisms might be able to produce a "living light" efficiently and sustainably.[44]

Most of the potential uses of synthetic biology for energy production and management are in the basic research phase. A conference sponsored by the Basic Research Directorate within the Department of Defense (Research and Engineering) in 2018 explored "Future Directions of Synthetic Biology for Energy and Power," and considered basic research directions involving electrocatalysis, electron storage (batteries), and ion transport materials.[45] Each of these directions can support application areas discussed above. The workshop concluded that the applications are varied and promising, but that much development will be needed to translate the scientific principles into practical applications.

Information Technology

While there are no extant computers based on synthetic biology, it is within the realm of possibility and a logical development based on a fundamental understanding of biological principles.

This discussion is not about attempting to mimic the human brain or simulate intelligence through the workings of neurons. Instead, we look at the functions of DNA editing, protein production, and metabolite synthesis as information processing tools and consider whether the storage and logic functions that are implied by such biochemical processes could be used for information technology purposes.

DNA is highly stable due to its unique chemical structure and the double helix structure formed by Watson-Crick base-pairing. Each position within a given strand of DNA may contain any one of the four natural nucleotides, adenine, thymine, cytosine, or guanine (an alphabet which could be expanded with new nucleotide insertions). Each position within a stand, therefore, is equivalent to two bits (or more) in a traditional binary computer. DNA can thus be viewed as an extremely compact, stable long term information storage system.[46] Then, transcribing DNA to RNA and translating RNA into protein can be viewed as a readout function, using random access addressing. Yet more relevant to computing, the ability to edit individual base pairs through gene editing techniques (such as CRISPR) represents state transitions according to specific rules, which is analogous to the processing of a Turing machine. However, with DNA processing, it is possible to perform multiple operations at the same time (parallel computing), and thus, biological computers could represent an entirely different model of computation.[47]

These ideas have been codified in a subfield called computational synthetic biology (CSB) which has been called "the next big thing in data science."[48] Companies have been forming in conjunction with investors, with early emphasis on data storage using DNA.[49] However, biology-based computer architectures might well be on the forefront.

Summary

In each of the application areas discussed above, there are practical ideas for producing products, and in many cases, nascent products being produced by companies. However, we have just begun to explore the potential applications of synthetic biology. Many new products and applications are possible. We are limited only by our current imagination and resources. We have likely not exhausted the areas of potential application here, having highlighted just six of today's most promising categories. New categories are likely to emerge, as additional synthetic biology tools become commonplace. New and yet more precise gene editing capabilities are likely to emerge. New protein and nucleic acid building blocks might be developed. A better understanding of how the genetic code is transformed into multicellular structures can lead to new structural materials. Our ever-increasing understanding of protein structures and properties as a function of the genetic sequence make extensions beyond nature-provided substances increasingly plausible. At this point, there is more that is possible than there is knowledge about genes and protein properties.

At issue is who will dominate in these fields, and who will be first to bring capabilities to market. With wide dissemination of results in basic research, as is appropriate, first to market depends on the spirit and ingenuity of entrepreneurs.

Recognizing that there are dangers, especially when unleashing reproducing biological entities, the opportunities are nonetheless compelling. The possibility of performing human gene line editing is an unfortunate distraction at this point in time, but one that will require international cooperation on responsible policies. The principal impediments to synthetic biology today consist of imagination and resources. Imagination requires a corps of educated and invested people inventing products and developing the production processes, likely as part of academia, start-ups, and government labs. Much knowledge of how to utilize synthetic biology has yet to be attained. Often, developing products will be hard, requiring a great deal of knowledge and expertise in biology and other disciplines. Such efforts ultimately require significant investment, both financially and societally as we need a cadre of scientists and entrepreneurs with sufficient knowledge to implement applications at scale. The US led the world, and reaped the benefits, in the development of microelectronics during the 20th century. It would behoove us to do the same as the burgeoning field of synthetic biology continues to emerge.

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