Principles of synthetic biology pdf

Do you like biology, biotechnology or genetic engineering? Are you interested in it, engineering or design? Synthetic biology is an innovative field that brings together these and many other areas to create useful tools to solve everyday problems. This introductory course in synthetic biology begins with a brief overview of the field, then looks at more challenging but exciting concepts. You will learn to design your own biological regulatory circuits and think about how you can apply these circuits to the real problems we face today. From basic oscillators, rocking switches and band pass filters to more sophisticated circuits that rely on these devices, you'll learn what today's synthetic biologists are building and how these circuits can be used in interesting and new ways. Join us to explore the field of synthetic biology: its past, present and promising future! 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Although edX has applied for licenses from the U.S. Office of Foreign Assets Control (OFAC) to offer our courses to learners in these countries and regions, the licenses we have received are not broad enough to allow us to offer this course in all locations. EdX really regrets that U.S. sanctions prevent us from offering all our courses to everyone, no matter where they live. Welcome to the BioBuilder program! We are delighted that you want to bring the tools of synthetic biology into your classroom. Online, we have a variety of documents to help you get started, including some practical lab video tutorials, Microsoft PowerPoint slides, study program guides and lab worksheets. In this written manual, we introduce ideas that underlie synthetic biology, some key aspects of biology that are explored in the field and in BioBuilder laboratories, and some useful information to use as you run the experiments in the BioBuilder program. In this chapter, we introduce the basic concepts of synthetic biology, explain how it differs from traditional biochemistry and genetic engineering, and begin to explore some of the fundamental principles of engineering that can solve problems using synthetic biology. At the most basic level, synthetic biologists, or biobuilders, want to design living cells to do something useful; for example, treating a disease, detecting a toxic compound in the environment, or producing a valuable drug. As Figure 1-1 suggests, synthetic biologists get these results by modifying an organism's DNA so that it behaves according to specifications, as engineers say, basically, it does what the bioconstructor wants. We can think of cells as complex miniature plants. DNA provides instructions for making all the machines in the plant: proteins, other nucleic acids, multicomposing macromolecular complexes, and more. These machines then perform the work of the cell. The body's natural DNA allows the cell to meet its basic survival and reproduction needs. Synthetic biologists can modify the DNA of a cell so that the cell assumes new useful functions (Figure 1-2). We will talk more about how researchers modify an organism's DNA later in the chapter. Ultimately, synthetic biologists would like to be able to build specialized living organisms from scratch using designed DNA. The field is not there yet. Currently, most efforts involve modifying existing organizations rather than building new organizations to behave in new ways. Many of the challenges that synthetic biologists target can be met by other engineering disciplines, such as electro, chemistry or mechanical engineering, but synthetic biology solutions offer some unique advantages. Even more striking, cells can make copies of themselves. Cars can't copy themselves, you need a factory to build a car. In addition, some organisms can copy themselves incredibly quickly, even with minimal nutrients. For example, in the laboratory, E. coli bacteria can replicate and split in about 30 minutes. Therefore, synthetic biology is an attractive approach to producing large quantities of a specific product because we can grow a cell programmed relatively easily to meet large-scale production demands. The cells also serve as a physical factory for production, providing much of the bricks and mortar infrastructure that would be required by other engineering solutions to meet the same challenge. Finally, the use of fast-dividing cells also facilitates prototyping and testing, which are very important for the design cycle, which we will discuss more in a little later. Second, cells contain biological machinery to perform many complex tasks — specific chemical reactions, for example — that would be difficult, if not impossible, to perform otherwise. And, they do so with nanoscale precision that is difficult to replicate in any traditional manufacturing facility. In addition, when their nanoscale machines break, cells have mechanisms to repair themselves, at least to some extent, which puts them at a great advantage over more typical plant-based production processes. Cellular complexity introduces its own to consider, too, but its potential usefulness is enormous. Third, synthetic biology has the potential to produce ecological solutions to many difficult problems. By necessity, the by-products of synthetic biology applications are generally non-toxic, because most toxic compounds would kill the very cells that do the work. In addition, the exploitation of natural cellular systems often results in economic processes. Current industrial production of compounds consumes large amounts of energy, often creating large amounts of waste that is harmful to the environment and frequently requires high temperatures or pressures. Beyond its usefulness in meeting real-world challenges, synthetic biology is also a fantastic approach to learning more about how natural systems work. As researchers dissect increasingly complex cellular functions, they can use synthetic biology to test their hypotheses from additional angles. For example, if their biochemical research results suggest that a certain protein acts as a kind of on/off switch, they can test this result by replacing the existing protein with a protein that is known to exhibit on/off behavior. If the new synthetic system and the natural system behave in the same way, the result provides further evidence that the natural protein acts as the researchers suspected. You may be wondering: do we know enough about cells to design them reliably, and if not, should we really try? There are many justifiable fears and concerns specific to synthetic biology. While other inventions such as the light bulb and the telegraph have been designed without a full understanding of the physics of electricity, engineering life has additional practical, moral and ethical challenges beyond those encountered in traditional engineering fields. For example, evolution can mutate DNA that has been meticulously programmed, ruining the engineering function of a cell. The replication of synthetic cells in the environment can be dangerous if they interact unexpectedly with existing organisms in this ecosystem. And, synthetic biology raises philosophical questions that we begin to think of cells as tiny living machines built to make our tender. Any technology that requires us to reconsider our interaction with the natural world must be approached with care. Researchers, bioethicists and government agencies are actively discussing these issues and working to develop synthetic biology responsibly that will improve the living world. We explore these issues in the fundamentals of bioethics. We are still in the early days of this developing discipline. As described above, synthetic biologists are not yet able to make organisms from scratch; currently, they work primarily in existing organizations. In addition, research has so far been conducted mainly on relatively simple single-celled organisms such as bacteria (particularly E. coli) and yeast (S. cerevisiae), although there is also some early success in more complex systems such as plants and mammalian cells. As the field develops, increasingly complex engineering will further expand the applications and potential benefits of synthetic biology. The synthetic biology approach might remind you of genetic engineering, in which researchers make small-scale rational changes to an organism's genome, such as eliminating a gene from a mouse or adding a human gene to a fruit fly, to study the behavior of the system. Synthetic biologists use many of the same tools as genetic engineers, as we will discuss in more detail later, but synthetic biology and genetic engineering differ in the scale at which they aim to make these changes. Genetic engineers usually introduce one or two small changes to study a specific system, while synthetic biologists aim to design new genomes and redraw existing genomes on a large scale. An illustrative, if fanciful, example of the potential scale of synthetic biology is the genetic reprogramming of a tree to transform into a fully functional home based on genetic instructions designed by a synthetic biologist. Such a system would take advantage of the tree's natural program (to grow by taking some nutrients from the environment) and use it for the needs of society. The genetic programming of a tree to become a home, however, is well beyond the scale of traditional genetic engineering as well as the ability of synthetic biology at this point. To achieve such large-scale design goals, synthetic biologists establish a structured engineering and design discipline, the principles of which we will introduce in the next section. Synthetic biologists also draw on the rich knowledge about how biological systems work that biochemists, molecular biologists and geneticists have obtained over the years. Specifically, scientific research has resulted in: reasonably well-characterized model systems, such as E. coli, yeast, algae and various types of mammalian cell culture, which provide a solid basis for exploring synthetic biology Abundant sequence data from a wide range of organisms, including bacteria, humans, mosquitoes, chickens, lions, mice , and much more, as well as tools for the comparison and analysis of sequences Molecular tools to move, reorganize and synthesize DNA to create new sequences Synthetic biologists use these discoveries and successes as the basis for which they apply an engineering mindset to solve real problems. The interdisciplinary nature of synthetic biology is suggested in Figure 1-3. Engineers build complex systems that must behave consistently, according to design specifications. To achieve their goals, engineers go through the design, construction and testing phases, often performing rapid prototyping of different designs to find the most promising direction. This procedure resembles the scientific method, in which the researcher cycles through hypotheses, hypotheses, and analysis. The main difference is that the scientific method aims to understand the precise details of how something works, while the engineering approach will not focus on why a design works as long as the prototype successfully tests. These differences are discussed further in depth in the Fundamentals chapter of Biodesign. Here we introduce a very simple example to show how different types of engineers could solve a problem: watering houseplants. By examining how different engineering disciplines could solve this problem, we will introduce some design fundamentals and illustrate how synthetic biologists apply a similar mindset and approach. Some people naturally have a green thumb, but others need extra help; otherwise, their plants end up looking dried and shrivelled. Different types of engineers would approach this problem of watering plants differently, depending on their expertise. For example, a mechanical engineer may design a pot with an unevenly weighted round bottom. When the tank in the bottom is full of water, it acts as a counterweight and keeps the pot upright. As the plant absorbs water, the counterweight decreases and the pot begins to tip over. This visual indicator would be an obvious reminder to the owner that the plant needs water. Maybe the leaning plant could even turn on a faucet to water itself. By putting technical feedback in the system, the pot would be on when the plant was watered, creating a closed loop control system. A potential complication with this design is that some plants need more water than others, so designers might need to create many different pots with different weights in the bottom, and gardeners would need to make sure they buy the correct pot for their plant. These types of considerations are an integral part of the design process. No design is perfect, and it is important to understand the strengths and limitations of any proposed design when considering the best way to proceed. An electrical engineer could find a completely different solution to the watering problem, one involving electrical moisture sensors and automatic watering. Its system can consist of many electronic parts: wires, resistances, capacitors, moisture sensors, circuit boards, and more. The various parties could work together to monitor the system, determine when plants need water, and then deliver that water when needed. This electrical engineering solution requires standardization, a crucial principle in all areas of engineering and principle that we will come back to later in this chapter. In this example of plant watering, each standardized electronic component was defined by the particular independent function it could perform. The components were built to meet a set of industry standards. This standardization of the basic parts allows them to be connected to each other easily and reliably, without the context affecting their behavior. These standardizations design, allowing engineers to know how a certain part will behave and how it can be combined with other parts to produce a desired result. It also simplifies manufacturing, allowing factories to produce millions of identical strengths for millions of different products. Synthetic biology has not yet reached this level of standardization, but it is trying to move in that direction. These two examples of traditional engineering solutions to the plant watering challenge illustrate how multiple designs can be used to solve even a relatively simple problem. The approaches were largely dictated and influenced by the toolbox available in each engineering discipline. Generally speaking, each approach is inspired by a toolbox with a few different parts, such as nuts and bolts that need to be put together, as well as a handful of methods to put things together, such as hammers and screwdrivers to assemble parts. The toolkit also contains concepts and ideas that guide each area. The specific elements of a toolkit tend to vary a little from one discipline to another. For example, the mechanical engineer's toolbox contains materials with a variety of properties, such as metal, plastic and concrete, as well as tools and methods to handle materials, including saws and welders. Gravity is an example of a concept they use in their designs. Electrical engineers, on the other hand, have a completely different toolbox. Their parts include wires, resistors, capacitors and circuits, and they have developed their own highly specialized manufacturing processes to create and combine these parts. Electrical engineering ideas use more of a modern understanding of electrical signals. For synthetic biology to become a mature engineering discipline, synthetic biologists need to define their toolbox. Like mechanical engineering and electrical engineering, the tools will include parts that need to be assembled and assembly methods; of course, the parts and methods will be specific to biology. Many of the tools in the synthetic biology toolkit are derived from molecular biology. In the next section, we will present some of the components of these existing toolkits and explore how they are also implemented in the synthetic biology toolkit. To explore the synthetic biology toolkit, let's first think about how biologists might approach the challenge of watering plants. Overall, they would use genetic tools to change the plants themselves. Such an approach could take Forms. For example, a solution could use a gene discovered in chameleons that is responsible for changing color in response to stress. It is possible that this gene can be inserted into plants; so they could then change their color to alert us when they need water. This approach is analogous to the mechanical engineer's approach of adding a visual indicator (the tipping pot) to help the owner remember when the plant needs There could also be a biological solution that is more analogous to the electrical engineering solution, which frees the plant owner from the need to provide water to all. What if it were possible to isolate a gene or two from a cactus plant — or, perhaps even more fancifully, from a camel — that helps these organisms withstand the very low water supply of their desert habitats? These genes, inserted into a plant, could help them survive with very little water, as well. These two solutions could be addressed with today's molecular biology tools, but these types of small modifications do not meet the goal of the synthetic biologist of genomic manipulation on a larger scale that would be necessary for an application such as growing a house and all its furniture from a seed. Such a wholesale genomic design requires a complete engineering toolbox. Such a toolkit must begin with and build on the contributions of established fields of molecular biology and genetic engineering. Although these methods have been around for many years and have been used for great effect in research, they are not sufficient for synthetic biology. They might be enough to insert a gene from a chameleon into a plant, for example, but they would not allow reliable reprogramming of a plant to grow in a two-bedroom, two-bathroom house. Therefore, we use the term genetic engineering, not synthetic biology, to refer to the relatively small-scale manipulation of genes in a host organism, perhaps by modifying at most a handful of genes. Synthetic biology, on the other hand, aspires to write and rewrite entire genetic programs to create useful functions and products. To achieve these more ambitious engineering goals, synthetic biologists are expanding their toolkit beyond traditional genetic engineering to include design principles of more established engineering disciplines. They are often inspired by the language of engineering, which provides a useful framework for thinking about design. These additional tools, which are still largely in development, include: standardization, abstraction and synthesis of de novo DNA. Standardization and abstraction are directly derived from the toolboxes of other engineering disciplines, while DNA synthesis is a unique engineering tool to synthetic biology. We will describe each of these topics in more detail later, but here are brief definitions: SYNTHESIS of DNA This is a process of chemical production of DNA strands without a pre-existing physical model, and is used at a much larger level in synthetic biology than is necessary for the Molecular. Standardization This is an approach that aims to generate a set of components that could be useful in multiple systems and that can be recombined for different results. Abstract This is a tool to manage detailed information when building a complex system. With it, designers can do the job without trying to keep in mind exactly how each detail of Works. In practice, engineers use different levels of abstraction depending on where they are in their design-build-test cycle. DNA can be produced by a series of simple chemical steps that are not fundamentally different from any set of chemical reactions that adds one building block to another. In the case of DNA, these building blocks are nucleotides, but other examples of polymers made from building blocks include proteins based on amino acids and polyethylene made from ethylene monomers. In a cell, DNA is synthesized using large macromolecular complexes that add each later nucleotide to the existing DNA strand. In the laboratory, chemists have developed other methods to produce DNA by chemically adding nucleotides to a growing chain of nucleotides. Whether manufactured in a cell or in the laboratory, synthesized DNA must have the right sequence. In a cell, the DNA sequence is based on an existing model flap that provides the sequence information. Synthetic biologists, on the other hand, often design new sequences for which there is no model. When there is no model flap to follow, they determine the nucleotide order of synthetic DNA using numerical sequence information. With this technology, synthetic biologists can write new DNA sequences that have never been written before. There are some limitations to the length of DNA strands that can be produced by this method, but a recent landmark has been reached with the synthesis of an entire functional genome by Dr. Craig Venter and his colleagues. This achievement simultaneously demonstrated the potential of chemical synthesis of DNA as a central component of the synthetic biology toolbox and raised ethical concerns about its use. Researchers reconstructed a genome of the bacterium M. mycoides using chemical synthesis to generate multiple short DNA extracts. They added a few small variations, which they called watermarks, to the sequence, and then inserted this synthetic DNA into a microbe (baker's yeast), where it was assembled into a complete genome. Finally, they transplanted the genome into M. capricolum, replacing the existing genomes of this bacterium and essentially converting M. capricolum shells into M. mycoides. This advance, which closely resembled Frankenstein's monster in Mary Shelley's famous work of fiction, stimulated the Presidential Commission for the Study of Bioethical Issues and led to a report, New Directions: The Ethics of Synthetic Biology and Emerging Technologies, which addresses the potential ethical issues associated with biology. mature DNA synthesis technologies. Standardization is a crucial part of any engineering discipline, as it facilitates the ability for designers to reuse parts, combine efforts with other teams and work effectively. For electrical engineering, such standardization means that designers can connect individual parts relatively easily so that they can arle to each other. For synthetic, synthetic biologists, allows DNA extracts to be physically and functionally connected. Physical assembly standards allow all pieces of DNA to be attached to other parts through a common strategy. This is similar to how mechanical engineers can connect any nut to any bolt because these parts all use standard sized wires. The complexity of the cellular environment and biological systems makes standard composition difficult. Nevertheless, there is an effort to set a standard for DNA assembly so that synthetic biologists have a collection of reliable parts and a place to find standardized genetic elements like promoters or repressors when they want to build with them. The physical normalization of DNA parts is discussed in more detail in the fundamental chapter of DNA engineering. However, successfully assembling parts is no guarantee that they will work as desired or interchangeable. Another consideration is functional standardization, which means that, regardless of the context, a genetic part will reliably encode a particular behavior. A synthetic biology approach to achieving this predictable functionality goal is the characterization of a cell's behavior in numerical terms: a DNA extract is either on (i.e. expressed by the cell) or off (not expressed). This numerical principle is familiar with all the electronics of our lives. Our TVs and cell phones are either on (even if they doragen) or turned off. This behavior all or not makes it relatively easy to connect different parts. When the TV receives the remote control input to turn on, it activates and provides a video and audio output. The same principle applies to the components that make up the electrical circuits: everyone receives inputs, either on or off, which determines its output, also on or off. This is a very simplistic description of circuits, but because the on and off states are standardized between parts, electrical engineers can connect parts and anticipate circuit behavior. Synthetic biologists are also trying to develop similar digital standards, describing a gene or enzyme as being activated or off. Of course, most biological behaviors (such as transcription or enzyme activity) are not completely digital, but the analogy holds up pretty well as long as we are careful. Using this approach, we can use other electrical engineering schemes, such as wiring diagrams and truth tables, to help us design our systems. These tools are described in more detail in the Fundamentals chapter of Biodesign. Through abstraction, biologists can design complex parts, devices and systems without worrying about every detail of how they work. Instead, the focus is on the end goal, which is the final output of the system or behavior. In practice, the design of any new system will use the levels of abstraction very naturally. At the beginning of the design process, we often think broadly about possible, possible, details of their implementation. As the problem and solutions are divided into smaller parts and become more defined, some of the previous abstractions become concrete so that we can actually build and test the designed system. Abstraction is particularly important for synthetic biology because the cellular environment and cellular processes are so complex. If we were trying to understand every detail of every new design, we would have to slog through our ideas too slowly. Instead, we can consider a bacterial cell as a black box (see Figure 1-5). In other words, we don't need to get bogged down in the details of each pathway within this cell, especially when developing initial designs. Figure 1-6 shows the hierarchical levels of abstraction. At the highest layer of abstraction is the system, our cellular black box. In this system, we might be interested in developing a device with a specific function such as detecting an environmental chemical and creating a specific output fragrance in response. When we decide how we want our device to work, we can start thinking about the different parts we will need to create each device; for example, a way to detect the chemical from the environment and a way for that response to control the production of perfume. Finally, at the lowest level of the hierarchy of abstraction — not at all abstract — are the actual genetic sequences that we will need to have on hand to use them as parts. By breaking the design process into these different layers of abstraction, we have divided the problem into bite-sized pieces that can be tackled more effectively. We will go into detail on each of these levels of abstraction and provide concrete examples of how to implement them in the design process in the fundamentals of the Biodesign chapter. In this chapter, we focused on the power of synthetic biology to produce new systems that can provide useful products or services. We introduced the basic concepts of synthetic biology by explaining how this field differs from traditional biochemistry and molecular biology, and how some of the fundamental principles of established engineering fields inform how synthetic biologists design and build living biotechnology. The engineering and design approach adopted by synthetic biology also has broader implications. As physicist Richard Feynman put it: What I can't create, I don't understand. Although we have certainly travelled a long way our understanding of biological systems, we cannot yet build entirely new systems. There is still much to learn about even the most basic biological processes and systems, and synthetic biology provides a powerful new tool in this business as well. Get BioBuilder now with O'Reilly online learning. O'Reilly members experience live online training, as well as books, videos and digital content from more than 200 publishers. Publishers. Publishers.