Chapter 1
What Is Synthetic Biology?

Abstract Synthetic biology aims at the design and construction of biological devices and systems for useful purposes. From an ideal engineering perspective synthetic biology works from rational design made through a few conceptual pillars, namely abstraction, standardization and modularity. Nevertheless, the combination of our still fragmentary biological knowledge and the messy nature of biological devices are major challenges for engineering life in a predictive manner. It is urgent to build bridges between different disciplines, from biology to engineer and back, to pursue this extraordinary goal of making life.

In an interview for a documentary film, the director of the Program on Emergent Technologies at MIT, Kenneth Oye, stressed: "the term Synthetic Biology seems to have been calculated to produce a negative reaction" (Schmidt and Meinhart 2009). True. Artificiality and nature do not combine well in most of our minds. And yet technology is one of the pillars of today's world and is without doubt one of the key factors behind the relative wealth and welfare of modern societies. Technology is also a powerful toolbox with which key global challenges such as climate change, food shortage or pollution issues can—hopefully—be tackled. Indeed, technology is both the cause of our historical success as a species and one of the best weapons for us to survive in the future.

In this book we will take a glance at one of the most promising new technologies: synthetic biology. But before we start, and in order to understand its very nature, we have to focus on its predecessor and sister discipline, biotechnology. In the vast range of relatively new technologies, modern biotechnology holds the world record for the fastest adoption. In less than two decades, transgenic crops, for example, have spread from their initial field tests prior to 1996 to their current overwhelming presence worldwide, with an enormous surface area dedicated to four main genetically modified crops today: soybean, cotton, corn and canola. Indeed, most of the cotton and corn on Earth are already transgenic cultivars.[1] But biotechnology is much more than transgenic plants. Genetically modified organisms produce drugs, synthesize biofuels, or carry out bioremediation. Biotechnology is

[1] International Service for the Acquisition of Agri-Biotech Applications (ISAAA). http://www.isaaa.org. Accessed 10 April 2014.

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defined by the UN Convention on Biological Diversity as "any technological application that uses biological systems, living organisms or derivatives thereof, to make or modify products or processes for specific use". This definition encompasses many applications that one would not consider biotechnological at first sight: baking, brewing and farming—including animal domestication and plant cultivation. All these are biotechnology because they use living organisms for a practical application (i.e., to make bread, beer or anything edible). In a strict sense, though, modern biotechnology is restricted to direct genetic modification of any organism for a practical purpose. Sometimes, but not always, genetic engineering is used synonymously with biotechnology. Bioprocess engineering, metabolic engineering or bioengineering are branches of biotechnology which are often perceived as a proxy of biotechnology itself.

1.1 Engineering Ideals and Synthetic Life

But if all these branches of modern biotechnology deal with engineering organisms, what about synthetic biology? The term "synthetic" (man-made) immediately suggests the antinomy of "natural". But synthesis (from the ancient Greek σύνθεσις, σύν "with" and θέσις "placing"; "placing together") is originally defined not by human manufacture but as the combination of two or more parts, either by design or by natural processes. Synthetic biology is thus an ambiguous term, which might be taken as either "artificial biology" or as "constructive biology"; there is certainly a little bit of both in the discipline. The most accepted definition of synthetic biology is the design and construction of biological devices and systems for useful purposes. This definition is very similar to that of modern biotechnology but, interestingly, terms such as "design", "construction", "devices", and "systems" call to mind the central feature that places synthetic biology somewhat apart from biotechnology: its combined—blended—nature between biology and technology (Fig. 1.1). The idea is as simple as it is appealing. Since there are no doubts about the intrinsic power of technological and engineering developments (bridges stand, cars run, pencils write, internet works and so on) such an engineering approach to molecular biology should make life easier to engineer compared to usual biotechnological approaches.

It has to be stressed here that biotechnology has been developed mainly by biologists, who, as is often the case in this classical discipline, deal with an almost infinite range of (biological) variants. In biotechnology, molecular cloning largely depends on trial and error, and biotechnologists—maybe imbued with the evolutionary dogma of the central role of selection—tend to make chimeric DNA on an experimental basis: if a DNA construction works, it is kept; otherwise, it is simply assumed that other variants have to be tested. But synthetic biology is not inspired by evolution but by engineering, and engineering works from rational design made through a few conceptual pillars, namely abstraction, standardization and modularity.

Abstraction stresses the need to streamline many processes (life is an extreme example of this). The idea begins by defining abstraction levels that do not need to

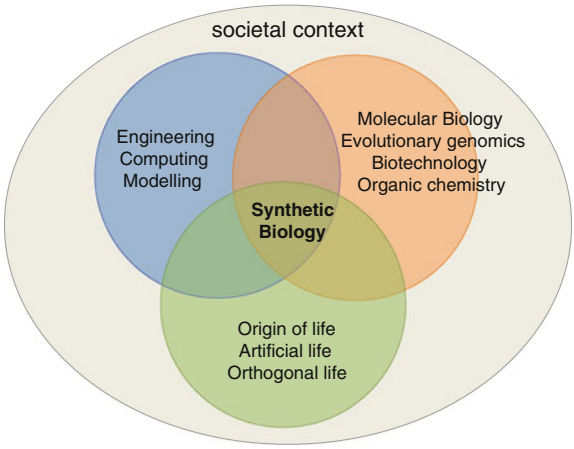

Fig. 1.1 Synthetic biology and its allied disciplines. Adapted from the European Science Foundation "Engineering Complex Biological Systems EuroSYNBIO" programme (Website accessed April 10th 2014 http://www.esf.org/index.php?id=5457)

be re-analyzed in every step of the construction process. Just as electric components are not demounted and tested individually but treated like black boxes (with the focus on the input/output rather on the particular internal workings), DNA cloning is proposed to make abstraction of the complexity (such as the DNA sequence itself; but also secondary structures, modulator interactions, etc.). With this approach, long complex sequences (ATGCTAGG…etc.) magically become biological parts, building blocks with which more complex associations (devices, circuits, etc.) can be mounted by the simple combination of individual parts.

These blocks, as in industrial engineering, are assumed standard. For instance, promoter sequences, i.e., pieces of DNA that modulate transcription initiation, can be scaled in a discrete range of strengths, like nuts and bolts. Thus, ideally, a synthetic biologist should be able to choose between a promoter of strength 5, 6 or 7, depending on the desired level of transcription. Promoters, reporter genes or pigment-coding sequences can thus be conceived as a palette (of colours, literally, in the case of pigments, or of strengths in the case of promoters) of defined strength/length/intensity/behaviour.

The combination of biological parts (a promoter of strength 7 and a fluorescent protein of strength 9, for example) might easily lead to devices or modules, which, in turn, are assumed standard and independent. Biological parts can be used as modules, that is, exchangeable blocks of defined function and useable in a range of microbial hosts and contexts. Modularity in biology is somehow different from the concept in industrial design, where modularity refers to the engineering technique that builds larger, more complex systems by combining smaller subsystems.

The concept of modularity is linked to orthogonality (etymologically, "straight angle"), which means independence. Two modules are orthogonal if they do not interact outside a defined interphase or, at least, do not interfere with each other.

This concept is central in engineering. Machines are designed and assembled in blocks, and these are exchangeable and do not interact with each other so as not to alter the overall behaviour. As we will discuss through this book, there is some debate among synthetic biologists as to the strict applicability of engineering constraints to biology. In the case of modularity and orthogonality, and considering the complexity and overlapping nature of metabolic pathways, could "relative orthogonality" be a realistic goal when engineering biology?

The particularities of life (complexity, flexibility, adaptability) represent both an obstacle and one of the main advantages of organisms compared to man-made machines. One feature present in every living form is emergence. Emergence was defined by philosopher G.H. Lewes, who wrote: "The emergent is unlike its components insofar as these are incommensurable, and it cannot be reduced to their sum or their difference". In other words, emergent properties are those that are not a direct outcome of the parts themselves; they are more than the sum of the parts. And this is something in conflict with engineering ideals. There is a famous quotation on emergence of one of the fathers of synthetic biology, Drew Endy. In an interview published in Edge,[2] he stressed: "I hate emergent properties. I like simplicity. I don't want the plane I take tomorrow to have some emergent property while it's flying." This is a very clear example of how biological complexity is a challenge for living beings to be engineered.

1.2 Challenges for Synthetic Life

Emergence is not the only challenge facing synthetic biology. There are both technical and organizational issues to be solved to firmly establish synthetic biology as the paradigm shift it is purported to be. In a review entitled "The ten grand challenges of synthetic biology" that one of us (MP) coauthored with other European researchers on the discipline, we defined a list of such challenges. These are logistic, technical and social (Table 1.1).

The first and the last challenges are social. The first requires the scientific community to reach a consensus on synthetic and streamlined genomes. It might not be obvious, but there are still difficulties in combining lexical and conceptual approaches from biologists and engineers and a common basis is required for the development of the discipline. The last challenge refers to science communication. After the disastrous Genetically Modified Organisms (GMO) battle, transparency should be one of the pillars of communication of synthetic biology if we want the discipline to be acceptable to the public. The remaining challenges are technical, and include difficult tasks such as modelling the complex and overlapping living circuitry or the challenge of combining engineering and selection-based approaches in the synthetic biology toolbox.

[2] http://edge.org/3rd_culture/endy08/endy08_index.html accessed April 10th, 2014.

Table 1.1 Challenges synthetic biologists have to deal with (from Porcar et al. 2011)

The ten grand challenges of synthetic life
1. Reaching a consensus on synthetic and streamlined genomes
2. Cooking from scratch (bottom-up)
3. Learning from nature: naturally evolved reduced genomes
4. Refine and make reality the notion of biological chassis
5. Manufacturing engineered biosystems
6. Overcoming physical and chemical constraints
7. From models to cells and back
8. Replication and reproduction
9. Towards an integrated design strategy of synthetic organisms
10. Coupling scientific development and public opinion information

Above all the challenges, there is the necessity to build bridges across disciplines (Delgado and Porcar 2013; Anonymous 2014). It is interesting to note that different visions coexist in synthetic biology today. Thus, there is no a unique synthetic biology approach, even less a unique definition or disciplinary borders. The different background of synthetic biologists mentioned above is at the basis of the controversial perception of the discipline, which is seen very differently from biological or engineering perspectives. Additionally, the first very ambitious predictions made on the success and applications of synthetic biology have been substituted by more realistic views, which are aware not only of the technical developments and milestones allowing more sophisticated, fast and relatively cheap genome modification, but also on the limitations of engineering life. There is still a controversy between the very conception of a living cell, which might be seen as a pure biological machine or as a radically different entity, arising from non-design and infested with emergent properties.

Background noise is a hot topic in many synthetic biologists' discussions. In engineering, a deviation of 0 and 1 might be due to noise in a strict sense, consequence of measurement bias, or correspond to loss of signal, such as in Internet data transfer. Often, values close to zero under a certain levels are corrected to zero; whereas values above a certain threshold are processed as ones. Background noise, interferences, signal loss and other factors mean that, even in the truly digital world, one and zero are not always pure values. In synthetic biology, promoter strength, fluorescent protein intensity, transcription force, enzyme activity and specificity, and many other factors have been quantified and scaled and assumed to behave in a scale from 0 to 1 or to 100. Compared to electronics or digital data transfer, biological processes are prone to avoid extreme values. Gene expression may be silenced, but there is often a certain leak. Biological noise—or messiness—is inherently associated to the nature of biochemical interactions, confined to soft chemistry in a nanometric scale. This important noise effect does not prevent biological circuits from working nor does it make biology impossible to engineer, but complicates the task of coupling and standardizing synthetic biological circuits enormously.

Mutation is one of the main features of life, and one of those that somehow sets biology apart from industrial production. Indeed, living creatures undergo various rates of random informational changes, which might have dramatic effects on adaptation, survival and evolution. Machines are designed to be more robust than flexible, and thus mutation-like processes do not usually occur. Interestingly, though, computer viruses, a source of "genotypic" (in computer science, hard drive) and "phenotypic" (computer behaviour) variation, work and spread very similarly to true viruses. Mutations, infections or—simply—time, always lead to the death of organisms. Machines are not immortal, but maybe it is farfetched to equate machine dysfunction with cell death because, for one thing, biological death is, by definition, irreversible, whereas machines can be repaired—by an external agent.

Extra-unit changes refer to the variation in the environment of a machine/cell. There are interesting similarities and differences between the relationship machines or cells have with their immediate environments. The former include the dependence of behaviour: for example many machines and bacteria work better at certain temperature ranges. Temperature, humidity, magnetic/trophic interferences, etc., are important factors in both biological and engineered systems.

It is certainly true that cells do not need to be biological machines to be genetically engineered. But it is also true that if a living cell is a very particular kind of machine, unveiling its complexity would rapidly lead to a fast and easy modification through standard engineering protocols. If one compares man-made machines and naturally occurring cells, several fundamental aspects are in contrast, and these include, at least: background noise and messiness, mutations and variability, unavoidable death, extra-unit changes, complex interactions with other units in a soft chemistry environment, a historical past (or phylogenetic dependency), and, last but not least, a particular internal organization that includes self-maintenance and self-repair, embodied in the fundamental notion of recursivity (see Chap. 3 for further discussion on cell-machine comparisons).

Machines are designed to fit and to stand with environmental conditions. In general they are made to work the same way on a very wide range of conditions. Cells, by contrast, flow with the environment and dramatically change their behaviour depending on that environment. Among the environmental factors with which interactions may occur, one of the most obvious is another unit (cell/machine). Sex is a revolutionary tool for evolution and adaptation, but undesired informational exchanges between computers, for example, are to be feared. The spread of computer virus above mentioned is in part due to a lack of prophylactic attitude when exchanging informational portable devices such as USB keys. The similarity of computer virus spreading with that of sexually transmitted diseases (STD) is striking, and it is certainly due to the fact that both processes share fitness selection as a blind but yet driving force.

Another issue that is troublesome is phylogenetic dependency. When the first modern biotechnologists attempted plant transformation, they had to modify and adapt bacterial or viral DNA sequences to make them "suitable" for plants. In fact, this phylogenetic dependency means a lack of standardization, and it has been compared—again—with computer operative systems. But a problem in the

metaphor is the number of such "operative systems" biological engineers have to deal with: even strains of subspecies might need important optimization and are recalcitrant to standard units (such as plasmids, for example). And, finally, a definition of the nature of life and the reason why the machine metaphor should be discarded: internal organization (recursivity, see Chap. 3). Cells exist, but since they have not been designed, they are built in a very different way compared to what an engineer would have done. Overlapping circuitry, complexity of interactions, emergent properties and a tendency to "do a lot with very little", or tinkering, clearly sets metabolic pathways apart from an engineering view of standard modules.

In summary, synthetic biology elicits diverse fundamental notions on living things and the possibility to engineer life. It is worth to mention that some practitioners, especially from the chemical field and referring to the history of their discipline, insist that synthesis is a research strategy and not a field, a strategy that enables us to explore problems, unveil discoveries and build new concepts in ways that observation and analysis cannot (see Chaps. 3 and 4).

References

Anonymous (2014) Beyond divisions. Editorial to a special issue on synthetic biology. Nature 509:151

Delgado A, Porcar M (2013) Designing *de novo*: interdisciplinary debates in synthetic biology. Syst Synth Biol 7:41–50

Porcar M, Danchin A, de Lorenzo V, Dos Santos VA, Krasnogor N, Rasmussen S, Moya A (2011) The ten grand challenges of synthetic life. Syst Synth Biol 5:1–9

Schmidt M, Meinhart C (2009) SYNBIOSAFE: Safety and Ethical Aspects of Synthetic Biology (DVD) Documentary Film, 38 minutes plus bonus material