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Nature *versus* design: synthetic biology or how to build a biological non-machine

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The engineering ideal of synthetic biology presupposes that organisms are composed of standard, interchangeable parts with a predictive behaviour. In one word, organisms are literally recognized as machines. Yet living objects are the result of evolutionary processes without any purposiveness, not of a design by external agents. Biological components show massive overlapping and functional degeneracy, standard-free complexity, intrinsic variation and context dependent performances. However, although organisms are not full-fledged machines, synthetic biologists may still be eager for machine-like behaviours from artificially modified biosystems.

Insight, innovation, integration

Synthetic biology (SB) includes an attempt to apply engineering principles to biotechnology. Although spectacular progress in metabolic engineering has taken place in the last decade, efforts to predictably modify organisms have only yielded limited successes. In the present article, we analyze one of the most popular paradigms of SynBio: the assumption that cells are machines. We focus on the differences between man-made machines and living systems and firmly conclude that cells are not machines, which has important theoretical and practical implications for the current development of SB. We suggest that further progress within the SB framework will be achieved by abandoning the bio-machine paradigm and by using an alliance between engineering and evolution as a guiding tool.

Synthetic biology in a nutshell

Contemporary synthetic biology (SB) is a group of different disciplines, from science and engineering, with motivations and ambitions ranging from fundamental studies on the origins of life and the understanding of minimal living systems to industrial applications of engineered cells.[1] Albeit the artificial synthesis of cells has been a scientific goal for more than a century,[2] nowadays scientists and engineers, driven by the major financial agencies, recognize themselves as SB practitioners when trying to model and rationally redesign cells within the engineering ideal framework of combining standard, modular, and orthogonal biological parts with a predictive behaviour.[3] Most of the time, these statements are made under the explicit assumption that cells and organisms are real machines[4] although, as we will discuss later in this paper, cells definitively are not machines and their components hardly follow the engineering ideal.

Expectations and achievements

The engineering view of SB claims that cells and organisms can be modified in a predictive manner by using standard, interchangeable parts. This idea contrasts with more classical biotechnology and metabolic engineering approaches, closer to trial-and-error strategies and that have dominated modern biotechnology based on DNA recombinant techniques. But how do the promises of engineering life compare to the real results? Taking the SB engineering premises literally (we can call it hard SB), it is difficult to identify all the main engineering concepts in living objects. Standardisation, one of the flagships of hard SB, faces the reality that, although there are important efforts to organize repositories of standard biological parts (*e.g.* BioBricks parts[5]), we do not have true standard components for a putative versatile combinatory repository allowing the assembly of new constructs with a predicted functionality in diverse biological contexts (on this topic, the reader is directed to a series of recent discussions and observations in the *Journal of Biological Engineering*[6,7]). A worldwide effort to build a community of users of standard parts through the international contest iGEM is very far from achieving its remarkable goal.[8] Regarding genome engineering, we are strictly in a phase of "genome plagiarism"[9] since there are too many unknowns in

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the field of genome structure, organization and regulation of gene expression to allow the writing of original genome sentences with a given projected function.

There is a plethora of promises and expectations in the name of hard SB, including improved industrial production of useful compounds such as drugs or biofuels, novel varieties of plants for a more sustainable agriculture, revolutionary personalized therapeutics, let alone unforeseen solutions for the problems derived from climate change.[10] The reality is that SB, in the sense of the application of engineering ideals to biology, offers remarkable potential for our understanding and use of biological systems,[11] but is still in its infancy.

Why organisms are not machines

The concept (or the metaphor, depending on the authors) of living beings as bio-machines is one of the most powerful *leitmotivs* of systems and SB. This is linked to the SB notion that any biological system can be seen as a combination of individual functional elements, as it is the case in man-made devices.[12,13]

Cells have been explicitly considered by synthetic biologists as particular kinds of (bio)mechanistic systems, such as Turing machines,[14] computers[4] or computers able to build more computers.[15] Interestingly, several efforts have been undertaken to make cells closer to this status. For example, Turing machine-like biosystems have been engineered to "build up a programmable peptidic sequence" in a rotaxane Turing machine for peptides;[16] or to allow them to "compute" synthetic biopolymers.[17] Likewise, a long-term goal of hard SB research is to build "biomolecule-based computers".[18] Interestingly, many SB reports contain statements such as "programming cells",[19] which can be considered as an indirect claim for a machine-like nature. Finally, the famous – and media-effective – quote on the first bacterium ruled by a synthetic chromosome which was given as "the first self-replicating species we've had on the planet whose parent is a computer"† can be considered as the apotheosis of the mechanistic view on organisms, either engineered or not.

It has to be highlighted, though, that the "machine nature of living beings", in most of these cases, is more a will as to what engineered cells should ideally be, rather than an ontological description of what natural living organisms actually are. This leads to an interesting point: are only engineered strains expected to behave like machines or will natural life forms also behave this way? We will come back to this issue later.

In his premonitory article "The biology of the future and the future of biology", S. Rose[20] wrote in 2002: "there has been a continuing tendency to understand living processes and systems by metaphorising them to the most advanced forms of current human artefact". However, in general, the community of synthetic biologists has either promoted or been tolerant with the machine status of cells. Opinions have emerged on the misuse of engineering metaphors in SB[13] and on the need to combine design with evolution.[21,22] However, the sharpest criticism of the machine concept has not come from experimental scientists but from philosophers and theoretical biologists. See for instance a special section recently published in *Studies in History and Philosophy of Biological and Biomedical Sciences*.[23] From the view of most epistemologists, the key difference between machines and organisms is purposiveness: the purposiveness of machines is extrinsic (they are designed and maintained by someone) whereas the purposiveness of organisms is intrinsic (they have not been designed and work in the absence of external intention).[24] Thus, all human machines depend on external agents (humans or other machines) to design, construct and repair them. In thermodynamic terms, machines are open systems for the flux of matter and energy. This is the only similarity with cells, in that they are also open systems for matter and energy fluxes. However, the fundamental difference between a machine and an organism is that the second one is a complex system "closed to efficient causation", to use the same words of theoretical biologist Robert Rosen.[25] Organisms are not designed systems, and internal causes, not external agents, drive their construction and repair. Ignoring this deep difference between machines and organisms could be a heavy constraint for SB since fabrication of cells and machines are two completely different things.[26]

We too, strongly argue that cells are not machines. In addition to the purposiveness principle stated above, which is a fundamental, ontological difference, we identify four theoretical and practical issues that keep living organisms far from man-made devices. These are:

1. Massive overlapping

Although plasmids or symbionts do exhibit a certain modular structure[27] it is unclear that organisms or metabolisms are fully composed of true modules: it is clear that they are characterized by anti-orthogonality. Orthogonality refers to the independence of behaviour and it is one of the pillars of industrial engineering. To be orthogonal, modules or, simply, parts, should not interact with each other outside well-defined interphases and should behave in a predictable way. In engineered systems, robustness is achieved through redundancy – replication of parts or circuits with the same function – whereas, in biology, functional overlapping and degeneracy are the rule, not the exception.[28] An outcome of the intrinsic flexibility of protein structures is the coexistence of diverse functionalities in the same protein: some of these functions are characterized as the principal, canonical, adaptive one, coincidental with other minor, non-adaptive performances, also known as promiscuous activities.[29] Although functional promiscuity could be considered as an annoying property from a SB perspective it is actually a source of evolutionary innovation in biological systems.[29] Functional promiscuity also adds unexpected interconnections to metabolic pathways.[30] Different behaviours and adaptations emerge from subtle variations of such interactions. Thus, it is not only a matter of the level of complexity of living systems compared to man-made ones – which could be solved by more powerful quantitative and modelling approaches – but the point is the nature of the "circuitry". Biology differs from engineering module-based devices by massive, changing,

† N. Wade, *New York Times*, May 20, 2010, "Researchers say they created a synthetic cell", http://www.nytimes.com/2010/05/21/science/21cell.html?_r=0 (accessed September 21st, 2015).

promiscuous interactions among almost all the components of a cell. While machines are designed to be formed by independent blocks that can be replaced, removed or introduced without massive effects on the whole system, in living organisms blocks are prone to functionally stick to each other.

2. Standard-free complexity

The number of operative systems in informatics is very low. In contrast, how many operative bio-systems are out there, in the biosphere? There are a huge number of species on our planet: approximately nine million eukaryotic species on Earth and in the oceans,[31] let alone bacterial diversity. From what we know in model microorganisms such as *Escherichia coli*, intra-specific strain-to-strain variations are huge,[7] so the number of "biological operative systems" is probably very high. It is true that biological parts such as viruses, transposons or plasmids do exhibit a wide exchange rate among different biological taxa (*via* infection, conjugation or horizontal gene transfer). This versatility should not be confounded with a standard behaviour, though. Firstly, the number of taxa compatible with a given virus or plasmid is just a fraction of the total. Secondly, tinkering *via* evolution is often needed to tame the exogenous DNA. And thirdly, the behaviour of the part is often very different in different organisms (or cell lines), which is in contradiction with the definition of standard.

As is the case for orthogonality, the key behind the reluctance of living systems to fulfil engineering principles relies on how complex systems originate: machines have been designed to be easily built from interchangeable, repairable parts; cells have not been designed but evolved, which has important implications in terms of part reuse and the (lack of) universal behaviour of the parts.

3. Intrinsic variation

One of the main features of life is its persistent tendency to change. Reproduction consists of making similar – not identical – copies of organisms. Recombination, genetic drift, horizontal gene transfer and, particularly, mutations, contribute towards the continuous non-directed modification of the genetic array of the species. The genomic variation of organisms within a given species is linked to their phenotypic variation, and the latter is linked to fitness, yielding the well-known scenario for natural selection to occur. Writing sentences or names in intergenic regions of a chemically synthesized chromosome was a good marketing strategy for the John C. Venter Institute's scientists, but from an evolutionary perspective was a mistaken choice, an ephemeral vanity mark, which will be rapidly erased by the high mutation rate of those genome regions, as Drew Endy has noted.‡

There are exceptions, though, of "non-designed design" in engineering, such as the popular example of the so-called automated antenna design with evolutionary algorithms, developed for NASA's Space Technology 5 (ST5) mission.[32] It has to be stressed though, that even in these cases, evolution-inspired engineering is restrained to the building stage, and yields a conventionally static "design" unable to further modifications that might display adaptive consequences (in engineering jargon, that might work better or worse). Not surprisingly, evolution-based approaches are increasingly used in synthetic biology, in the form of laboratory adaptive evolution (LAB), which basically corresponds to artificial selection, and directed evolution (a combination of rational design with purification rounds of selection).[33] This growing role of random variation and selection as a helping force for rational design has been graphically described as constituting "an interesting withdrawal from the machine-analogy that might indicate that maybe in the end the rational design of a living organism might be beyond human capacities."[34]

4. Context dependence and the outer world

For many biotechnology and SB experts, it is surprising the ease with which media-sound developments in bioengineering elicit strong negative reactions in the public. The complex reasons behind this fact are beyond the goals of this article, but we want to stress here one reason that is often overseen: the tendency to extrapolate the fitness of laboratory-developed microorganisms (obtained either through genetic engineering, selection processes or a combination of both) to the one they will have in the wild. Experienced biotechnologists know that this is almost never the case: there is no *prêt à porter* SB. This is basically due to varied and key differences between the lab and other, far more complex, environments (in terms of, mainly, but not restricted to: growth media and inter-specific, ecological interactions). For example, microorganisms used in bioremediation face an almost infinite number of possible ecologic inter-species interactions in natural ecosystems that are virtually impossible to model. Even in a much simpler scenario, such as industrial fermentations with axenic cultures, scaling up typically yields unpredictable results because of plasmid losses, metabolic shifts or variations in stress conditions, to cite a few.[35] Biological systems have evolved under complex ecological pressures. Synthetic organisms are designed in a simpler, controlled environment, which makes behaviour extrapolations very risky.

Conclusions: designing the non-designed?

Although cells are not machines, in the deep sense that they are not engineered in origin, they may yet become engineerable, that is, they can be modified in such a way that they at least partially fulfil engineering assumptions. The major success of the microbial synthesis of a precursor of the antimalarial drug artemisinin is a good example. By using both metabolic engineering and tinkering, very high artemisinic acid production in both *E. coli* and yeast have been obtained.[36] Does this mean that those microbes have been converted into drug-producing bio-machines? In the absence of complete predictability and

‡ Cited by C. Zimmer, 2008, "Frankestein was here": Synthetic biology as grafitti, http://scienceblogs.com/loom/2008/01/31/frankenstein-was-here-syntheti/ (accessed September 21st, 2015).

decoupling principles, they are not exactly bio-machines, but they do efficiently perform the task for which they have been, at least partially, designed. On the other hand, and since microbial synthesis of artemisinic acid was an overwhelming success that overtook the expectations of the project, does a definition of the nature of the transformed organism really matter?

As we have discussed above, flexibility (*i.e.* protein structure flexibility and promiscuous functions) is a basic trait of biological molecules, which is characterised by soft constituents with frequently changing shapes, rather than hard building blocks, such as actual Lego pieces. Engineering must also deal with flexibility, but usually to a lesser extent than biology. Interestingly, Lego, the paradigmatic assembly system inspiring SB, has a "soft Lego" division with more flexible blocks. However, the main goal is to avoid damage in small children rather that increasing the interaction network of each piece, which basically remains the same.§ In SB, recently, important efforts have been undertaken to either flexibilize engineering constraints,[37] to identify and reduce metabolic burden associated with heterologous gene expression,[38] or to combine rational design with evolution.[22] All those efforts might contribute towards a different conceptual framework in SB in which engineering principles will be used more as an inspiration than as imperative requisites for metabolic engineering to be considered successful. Directed evolution approaches, for example, by combining rational design with tinkering through evolution, constitute a very good approach for bioengineering, to make man-made designs – flexibly – fit in already-working biological systems. We strongly believe that merged rational-evolution scenarios will coincide with a new golden age for SB, offering new alternatives to make biology easier to engineer.

Finally, we would like to emphasize an important point. We are convinced that organisms are not machines but we do not assume that they exist due to a supernatural or non-natural reason: notwithstanding the efforts of the proponents of intelligent design, the days of vitalists are gone. Organisms exist because physical and chemical processes, passed through the filter of adaptation and evolution, build them from within. Of course, machines do follow physical laws as well. But these two premises cannot lead, *per se*, to the conclusion that cells are machines. In our view they are not – although, as synthetic biologists wish, they may become machine-like one day.

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§ https://education.lego.com/es-es/lego-education-product-database/preschool/45003-lego-soft-starter-set

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