Are we doing synthetic biology?

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Abstract Synthetic Biology is a singular, revolutionary scenario with a vast range of practical applications but, is SB research really based on engineering principles? Is it contributing to the artificial synthesis of life or using approaches “sophisticated” enough to fall outside the scope of biotechnology or metabolic engineering? We have reviewed the state of the art on synthetic biology and we conclude that most research projects actually describe an extension of metabolic engineering. We draw this conclusion because the complexity of living organisms, their tight dependence on evolution and our limited knowledge of the interactions between the molecules they are made of, actually make life difficult to engineer. We therefore propose the term *synthetic biology* should be used more sparingly.

Keywords Synthetic biology · Metabolic engineering · Artificial life

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What is synthetic biology?

Synthetic biology (SB) is an emerging research field with a simple, yet dual definition it seeks the design and construction of new biological parts, devices and systems, as well as the re-design of existing parts for useful purposes. This framework is vague enough to include modern biotechnology (the use of living organisms and bioprocesses in engineering, technology, medicine and other fields requiring bioproducts); however, there is general assent on the intrinsic nature of SB as a differentiated field compared to biotechnology, metabolic engineering and systems biology. It has been suggested that some aims of SB “would be difficult if not impossible to distinguish from more traditional engineering goals” (Keller 2009). A sociological study (Calvert and Fujimura 2011) shows that “a clearly stated aim of SB is to make biology into an engineering discipline” in “an attempt to make biology less qualitative and descriptive and more quantitative and predictive”. In parallel, SB is assumed to exhibit a characteristically higher degree of “sophistication” compared to metabolic engineering, for example.

Today, SB is perceived as a revolutionary scenario that will facilitate mass production of an array of useful compounds, ranging from drugs (Mitchell 2011) to biofuels (Connor and Atsumi 2010); it will be key in the development of bioremediation (de Lorenzo 2008), increase crop yield (Blankenship et al. 2011), lead to the production of novel food ingredients and a large variety of chemicals (Erickson et al. 2011), and improve human health (Weber and Fussenegger 2011). The scientific production citing or reporting SB research has dramatically increased in recent years. For instance, a search run in the Scopus database for scientific documents including “synthetic biology” in their titles yields 11 documents for the period 1960–2000; 71 for

2001–2005; and 1,088 documents for 2006–2010 (accessed Jan 24th 2012). It has to be stressed, though, that is difficult to determine whether this exponential growth in the SB literature corresponds to an actual revolutionary expansion of the intrinsic potency of the discipline or just to a fashionable use of the term.

Synthetic biology and metabolic engineering

In 2006, it was reported the production of a precursor of artemisinin, an antimalarial drug, in engineered yeast (Ro et al. 2006). This important advance was the first step towards economical production of artemisinin from engineered microorganisms, including *Escherichia coli* (Anthony et al. 2009), in which, over a 4-year period, yield was increased more than 1 million-fold (Keasling 2010). The approach included three steps: engineering the native mevalonate pathway, inserting the amorphaadiene synthase gene, and cloning a novel cytochrome P450 mono-oxygenase gene from *Artemisia annua* (the plant from which “natural” artemisinin can be extracted). Thus, a complex synthetic pathway was implemented in order to optimize artemisinic acid production. Constant adjustments were needed, particularly in *E. coli*, mainly because of the toxicity of several of the intermediates involved (Keasling 2008). If the “sophistication” of a genetic modification is to be claimed as the reason for classifying a successful technical achievement as SB research, then microbial-based synthesis of useful compounds, as in the case of artemisinin, certainly can. However, if, beyond modelling, the engineering principles of predictability, lack of noise, orthogonality and standardization are mandatory for classification purposes, that major accomplishment would have to be considered as a particular—albeit wonderfully complex—exercise of metabolic engineering.

Genome writing or printing?

Another milestone in SB research and, by far, the one with the largest impact on the media was the astounding synthesis of a fully synthetic (in the original etymological sense of a combination of several entities that together form something new) bacterial chromosome (Gibson et al. 2008), which 2 years later proved fully functional (Gibson et al. 2010). This report demonstrated the potency of combining high-throughput DNA chemical synthesis and genome transplantation techniques, which might be key in the future development of synthetic organisms. The controversy sparked by that major technical accomplishment (Giuliani et al. 2011; Bedau et al. 2010) raises an interesting point that deserves further attention: the concept

“writing” DNA. It is obvious that the ability to synthesize DNA at one’s will strongly recalls human writing, and this is certainly behind the success of the metaphor: synthesizing DNA is assumed to be “writing” in DNA language. It has to be noted, though, that genomes are made of DNA sequences that are either of unknown function, poorly understood or code for proteins whose interactions are not well characterized. Chemical synthesis can thus accomplish “printing” (copying) whole genomes with a few, evolutionarily short-lived, modifications such as watermarks in the intergenic sequences; but the term “writing” might be best suited for intentional synthesis of fully characterized artificial DNA assemblies of predictable behaviour. Thus, despite the new possibilities opened up by full genome-scale synthesis methods, we might not be in the DNA writing era yet.

Synthetic metaphors and living machines

Synthetic Biology is particularly rich in all kinds of other metaphors, such as chassis and orthogonality, the perilous use of which has recently been recently stretched (de Lorenzo 2011). The concept of chassis, borrowed from vehicle engineering, refers to a simplified—yet to be developed—synthetic or semi-synthetic cell onto which multiple modules with specific functions can be mounted. These modules—devices in the SB terminology—are expected to behave in an orthogonal (independent from each other and from the chassis) way. It is doubtful whether typically promiscuous biological circuits can work orthogonally, and it is certain that we are still far from having a real chassis. Even if one of the main SB goals is to make life easier to engineer (Endy 2005), the simplest cell is still too complex to be rebuilt from scratch or fully re-engineered. The many outstanding reports listed in the reference section of this article bear witness to the increasing success of genetic engineering. However, these successes tend to be recalcitrant to SB principles of standardization and orthogonality. One of the most influential reports on the SB community illustrates these limitations: refactoring phage T7 is the first example of rational redesign of a full genome (Chan et al. 2005). Nevertheless, it is concluded that the vast majority of the rationally introduced genomic rearrangements yielded a lower biological fitness (made smaller lysis plaques) than the wild-type strains. Another recent example of our incapacity to accurately predict the behaviour of even the simplest biological system is the failure of the rational approach for experimental evolution in model animals. Virologists have tried to identify avian flu virus mutations involved in the ability for contagion between humans. Do the results with ferrets (the experimental model of choice) apply to

humans? The affirmative supposition has been at the very centre of all the societal and political debate on this controversial research (Cohen 2012). The unpredictability of living systems circuitry is both a consequence of their inherent stochasticity and of our incomplete knowledge of molecular interactions, which—unlike man-made machines—are prone to exhibit higher complexity than the sum of their parts (Moya et al. 2009; Letelier et al. 2011). Messiness in biology might not be a direct consequence of natural selection but the inevitable outcome of multifunctionality, promiscuity and flexibility of proteins, ingredients that also at their turn may serve as the raw matter for evolutionary opportunism and tinkering (Tawfik 2010). There is a famous quotation on this typically biological complexity “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” [Drew Endy, in an interview to *Edge* in 2008 (Anonymous 2008)]. Life is the outcome of evolution through—mainly—natural selection, which has resulted in what appears to be an intrinsic and universal feature of the living: a moderate number of parts, a high number of interactions among them and a tendency to overlapping of circuitry. And whereas a rationally-designed plane is an exact metaphor for what synthetic biologists would like life to be, the Delphic boat might be a more realistic—albeit poetic—view of what life actually is: “The oracle of Delphi had the habit of questioning passers-by One of the questions told the following story I have a boat made of wooden planks As time elapses they rot one after the other At some time no original plank still remains in the boat is it the same boat? Clearly the owner will say, yes and he will be right The boat is not the matter of the boat, but something else, much more interesting, that orders the matter of the planks it is the relationships between the planks” (Danchin 1998).

Standards in biology—the iGEM competition

The main aim of the international Genetically Engineered Machine competition is to motivate students to attempt to build simple biological systems from standard, interchangeable parts. In order to achieve this, each team is provided with a library of standardized (BioBrickTM) parts. Biobricks are supposed to make easier and faster cloning by a cut-paste strategy technically identical to standard molecular biology techniques, but yet with a radically novel goal: allowing infinite combination of biological parts in cell, in a Lego-like fashion. However, the superiority of Biobrick-based cloning compared to standard techniques is subjected to discussion, and their implementation is currently challenged by the dropping costs of DNA synthesis. iGEM teams can then either use these Biobricks to build GMOs or, alternatively, they can also submit their own BioBricks to the Registry of Standard

Biological Parts (<http://partsregistry.org>, accessed June 20th, 2012), a nonprofit organization that accumulates several thousands of BioBricks donated by research groups worldwide. The success of iGEM (the competition was split on 2011 into three regional phases and a MIT-based grand final because of massive participation) is a demonstration of “engineering ingenuity” (Goodman 2008) and many iGEM result in major publications. In general, though, successful, award-winning projects often avoid using previously characterized standard parts from the Registry and choose to design, characterize, use and submit new “standards” to the registry. For example, the six finalist teams of the 2010 iGEM competition submitted more than 300 new parts (half of which were submitted by the Grand Prize Winner, Slovenia), with only one team (BCCS Bristol) using parts from the registry to build their key Biobrick (team wikis available at http://igem.org/Team_Wikis?year=2010, accessed June 20th, 2012). Even more surprisingly, in the 2012 edition, Groningen, with a holistic and heterodox (screening-based and standard-free) strategy for the identification of promoters to identify volatiles from spoiled meat by microarray analysis, was awarded the Grand Prize. This reveals the difficulties of using in a particular biological system what others teams used in another.

Paths to synthetic life(s)

Beyond genome synthesis and redesign of metabolic networks for biotechnological purposes, there are two other pillars of SB, both directly aiming to construct a synthetic cell (Peretó and Català 2007). The top-down approach aims at simplifying already reduced cells—mainly endosymbionts or parasites—in order to yield a “chassis” for further SB devices to be mounted on; and the bottom-up approach, the ultimate goal of which is to construct a synthetic cell from scratch. The top-down approach strongly relies on evolution, since it takes advantage of existing naturally reduced cells on which to perform further artificial reduction (Gil et al. 2004; Moya et al. 2009). It should be noted, though, that the transcriptomic and proteomic complexity of naturally reduced genomes is highly intricate as shown by Güell et al. (2009) and Kühner et al. (2009). To date, this approach is predominantly evolutionary but its development will very likely be marked by selective approaches, since wherever the lower limit of complexity lies (and different minimal cells may well originate from different environmental conditions), it must stand within the boundaries marked by biological fitness.

The bottom-up approach is, without doubt, at the very centre of the interdisciplinary framework of SB. Of all SB pillars, this is the one more clearly resuming life’s origin

and has been proposed to provide novel perspectives on the origin of life (Malaterre 2009; Dzieciol and Mann 2012). The bottom-up approach strongly relies on selection and evolution and this will become more obvious as the achievements of this “cooking from scratch” strategy progress (Porcar et al. 2011). In fact, some theoretical and experimental approaches to chemistry-based protocell biology converge to standard SB (Solé et al. 2007). Interestingly, Kurihara et al. (2011) recently reported the first link between giant vesicle self-reproduction and the amplification of encapsulated DNA. This work shows that, as a consequence of physical interactions of the PCR-amplified DNA with the surrounding membrane components, vesicle reproduction is stimulated. This is the first time that self-replication of an informational substance (DNA) has been linked to self-reproduction of the compartment containing the information (the vesicle). Also for the first time, a very primitive natural-like selection can be considered to be taking place in this artificial construction, since DNA determines the “biological fitness” (in terms of “reproduction”) of the vesicles.

Lying somewhere in between bottom-up and top-down approaches, xenobiology is an emerging research topic aiming at the construction of functional alternative nucleic acids. After initial failures (Benner et al. 2011) and by using non-deoxyribose sugars, alternative genetic polymers based on simple nucleic acid architectures not found in nature, xeno-nucleic acids (XNAs) capable of evolution have been recently reported (Pinheiro et al. 2012). The expansion of the genetic code has also successfully been achieved, allowing, for example, the specific incorporation of amino acids not encoded in the genetic by an *E. coli* strain harbouring a Sep (O-Phosphoserine) accepting transfer RNA (Park et al. 2011).

Interestingly, this “chemically parallel life” is an emerging research field that was first proposed as the ultimate biosafety tool, mainly because of its supposed incompatibility with standard DNA- and RNA-based life (Schmidt 2010), but concerns have now arisen on the possibility of interactions of xeno-organisms with today’s living forms (Joyce 2012).

Conclusions

Engineering is defined as the science, skill, and profession of acquiring and applying scientific, economic, social, and practical knowledge, in order to design and also build structures, machines, devices, systems, materials and processes. The key concept in engineering is design, and this is also the point for SB: design life. We believe that, it is actually difficult to tell between synthetic biology, metabolic engineering and biotechnology reports. Our reasons

lie in the particular complexity of living organisms, their tight dependence on evolution and our limited knowledge of the interactions of the molecules they are made of, which together actually make life difficult to engineer. We propose the term Synthetic Biology to be used more sparingly, restricted to four research fields: (1) Model-inspired research following strict engineering principles applied to biotechnology; (2) Top-down approaches significantly simplifying cell complexity and aiming to implement semi-synthetic cells that are easier to manipulate further (in a broad sense, a *chassis*); (3) Bottom-up, mostly empirical explorations aiming at de novo construction of increasingly complex proto-cells, displaying a link between genotype (informational substance) and phenotype/behaviour (e.g., vesicle division efficiency); and (4) xenobiology research aiming at developing novel organisms with chemically alternative nucleic acids as information biopolymers, an extended genetic code or a larger set of amino acids. As exclusion criteria we identify the following: lack of design, use of assay/error tuning strategies, and lack of orthogonality and/or modularity. Research with those features should not be considered SB as ad-hoc strategies and standard-free approaches are incompatible with canonical engineering.

Interestingly, many recent assays claim for the need to develop a brand-new evolution-based approach to SB (Rothschild 2010; Porcar 2010; Schwillie 2011). As long as engineering, bottom-up and top-down approaches converge to develop truly artificial cells, this assisted design might prove essential, since life—as we know it—cannot be prevented from evolving.

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