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Review

Biological standards for the Knowledge-Based BioEconomy: What is at stake

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ABSTRACT

The contribution of life sciences to the Knowledge-Based Bioeconomy (KBBE) asks for the transition of contemporary, gene-based biotechnology from being a trial-and-error endeavour to becoming an authentic branch of engineering. One requisite to this end is the need for standards to measure and represent accurately biological functions, along with languages for data description and exchange. However, the inherent complexity of biological systems and the lack of quantitative tradition in the field have largely curbed this enterprise. Fortunately, the onset of systems and synthetic biology has emphasized the need for standards not only to manage *omics* data, but also to increase reproducibility and provide the means of engineering living systems in earnest. Some domains of biotechnology can be easily standardized (e.g. physical composition of DNA sequences, tools for genome editing, languages to encode workflows), while others might be standardized with some dedicated research (e.g. biological metrology, operative systems for bio-programming cells) and finally others will require a considerable effort, e.g. defining the rules that allow functional composition of biological activities. Despite difficulties, these are worthy attempts, as the history of technology shows that those who set/adopt standards gain a competitive advantage over those who do not.

Contents

Introduction

The onset of DNA cloning in the late 1970s marks a historical boundary between pre-scientific, experience-based biotechnology and the science-based modern version that we enjoy today. The latter largely depends on the capacity to access genetic

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information from given organisms and modify it almost entirely at will. The aims include the production of biomolecules or biomaterials, as well as delivery of biological activities of interest, in a time and space different from how they appear naturally. Shortly after its early development, the whole of DNA-handling technologies to this end were grouped under the overarching term *genetic engineering* [1]. This term, which was quickly and universally adopted, evokes an agenda of building biological systems following a rational logic similar to what engineers do when constructing complex objects. But despite the powerful metaphor, what we call to this day genetic engineering has in reality very little to do with *bona fide* engineering [2]. Instead, what the field has been doing for the most part is more similar to what one could call genetic *bricolage:* on the basis of spare parts about which we have limited knowledge, we connect them in various ways with a limited logic and try to make them work. What has been achieved thus far with even such a simple trial-and error approach is impressive, but by no means the end of the story.

The onset of systems and synthetic biology and their emphasis on rigorous quantification and description of biological objects in their whole multi-scale complexity has raised the opportunity to look at living entities through an authentic (not just a metaphoric) engineering perspective [3]. This view stresses the cataloguing of the systems' components, the relational logic that makes them work as they do and the definition of the boundaries between the different organizational levels and modules. Under this framework, the agenda of the modern biotechnology that builds on systems and synthetic biology is to make the design of living objects an authentic engineering discipline [4–6]. This asks to bring to the biological realm questions and criteria that have been generally alien to life sciences research. Six of them can be immediately identified: [i] rules for physical and functional assembly of components into higher-order systems; [ii] metrology i.e. units describing biological structures and activities and ways to measure them; [iii] retroactivity/context sensitivity i.e. influence of the engineered implant in a biological chassis and *vice versa;* [iv] uniform descriptive language to report biological properties qualitatively and quantitatively; [v] storing and managing information; and [vi] risk assessment. The dividends of raising standards for each of these aspects are diverse and support the aims of the Knowledge-based BioEconomy—KBBE [7–9] and the 4th industrial revolution at large [10. Standards do sacrifice flexibility and limit the freedom to operate but gain enormous advantages in efficiency and reproducibility.

Why standards?

When different communities wish to work together they need to adopt standards that enable their interplay in time and space (Table 1). Standards allow decoupling of design from production from assembly from deployment—and they help to reduce the lack of reproducibility of results that plagues the scientific and technical literature in biology and biotechnology [11]. A separate and by no means minor aspect is that of leadership (https://goo.gl/h9ptfX). The very word *standard* evokes the notion of a group following someone with a banner [12]. The history of technology has numerous examples of how those who developed well-grounded standards at the right time gained competitive advantages that were later followed by many others. In other cases, standards have a clear political angle that has to be solved through either imposition (e.g. adoption of the metric system during the French Revolution) or negotiation [13,14].

But what can be the subject of standardization efforts at this time in the biotechnological domain? The majority of the attempts to tackle this issue have focused thus far on bacteria (Table 2). They are the biological systems of immediate biotechnological value that are more amenable to deep genetic engineering with the technologies we have at hand now. Note, however, that bacteria are being rapidly caught up by yeast [15] and plants [16] as biological *chassis* amenable to sound bio-programming. Unfortunately, the developments in that field are outside the scope of this short article. Note also that a separate, vibrant branch of current biotechnological research tackles cell-free systems [17,18] in which the components of the bioprocesses of interest are extracted or enriched from their biological origin and recreated in an *in vitro* setup. These systems are far more predictable and easier to engineer than their *in vivo* counterparts. They have been the subject of different and successful standardization efforts, as they

Table 1
What is involved in standardization.

Standardization subject	Standardization challenge
Physical assembly of system components	Definition of geometrical shapes
	Specification of dimensions
	Compatibility of boundaries between elements
	Compositional rules
Functional assembly of system components	Quantitative description and management of context-sensitivity
Metrology	Units of measurement of relevant properties
	Conditions and procedures to calculate units
	Reference values and objects
	Tolerance and allowance
	Context sensitivity
	Transfer functions
Handling/manufacture of engineered objects	Standard operating procedures (SOPs)
	ISO standards
Formal languages	System description
	Workflow description
	Data exchange
	Programming (operative systems)
Databases	Spread sheets/work sheets
	Metadata
	Interoperability/Compatibility
Risk assessment	Safety criteria
	Security benchmarks
Ethical appraisal	Consensus rules
Promulgation	Enforcement
Intellectual property management	Patents, Open Access, Open Source [75,76]

Table 2
Standard and standard-izable microbial chassis for the KBBE.

Genus/species	Qualities of interest	References
Mycoplasma sp.	Small genome, vehicle for delivering therapeutic activities to the lung	[26]
Escherichia coli	Laboratory work horse, recombinant DNA host, abundant genetic tools	[77]
Pseudomonas putida	Tolerance to environmental insults (solvents, redox stress), platform for metabolic engineering	[78,79]
Bacillus subtillis	Laboratory workhorse, easy recombineering, efficient secretion systems	[80]
Corynebacterium sp.	Long time applications in industrial biotechnology, large-scale production of amino acids	[81,82]
Saccharomyces cerevisiae	Laboratory workhorse, easy genetic manipulations, optimal eukaryotic metabolic engineering platform	[82]
Synechocystis/Synechococcus	Photosynthetic organisms, CO2 fixation, emerging metabolic engineering	[83,84]
Streptomyces sp.	Diverse secondary metabolism, production of antibiotics, efficient secretion systems	[85]
Vibrio natriegens	Super-rapid growth, easy to engineer, host of recombinant DNA constructs.	[86,87]

are very useful for rapid prototyping of genetic circuits [19]. But cell-free systems lack (at least for now) the scalability and self-replication properties of whole cells that make living micro-organisms so appealing for industrial applications.

On this background, we inspect below some outstanding scenarios of elaboration of shared rules for measuring activities, assembling components, formalizing context dependency and describing rigorously events in the biological realm. Our main argument is that adoption of standards is bound to accelerate the transition between contemporary genetic engineering-based biotechnology and the future bio-engineering-based KBBE. In particular, we substantiate how standards may bring bacterial biotechnology to an unprecedented level of efficiency and reliability that will make a difference to the onset of the 4th industrial revolution.

Engineering meets biology—in earnest

A least 4 branches of engineering are useful as conceptual frameworks to both understand and reshape biological objects with a biotechnological purpose. First *mechanical engineering*: if human-made setups work as they do it is because their design follows a relational logic and a connectivity between their (physical, chemical) and material components that can be [i] rigorously subjected to hierarchical abstraction before their implementation and [ii] eventually deployed owing to the compatibility between their boundaries. Second, *electrical engineering*: most devices that form part of daily life are run by electricity, both as the source of energy and as the signal carrier through a given, multi-scale system. A number of well-established physical variables (e.g. current, resistance, voltage, *etc*) and units (amperes, ohms, volts) describe electricity and allow a faithful description of any electric or electronic circuit. Third, *computer engineering*, that deals with the construction of both hardware and software and enables the programming of the resulting whole to run complex calculations and execute given actions e.g. through sensors and actuators (Fig. 1). One key outlet within this realm is *information technology*, which focuses on tools (e.g. statistics and other mathematical methods and algorithms) to process data, enable decision-making, identify computability and run higher-order simulations. Finally, *(bio)chemical engineering* focuses on the buildup and operation of efficient factories to transform feedstocks into products that are both technically viable and economically competitive. These branches of engineering often converge in devices (e.g. portable PCs, tablets, smartphones, *etc*) and products (e.g. advanced drugs or materials) that characterize contemporary lifestyles. But seen in retrospect, it was the *domestication* of electricity as a source of energy and its exploitation as a signal carrier in information technology which made the biggest difference to our contemporary ability to design and produce objects and molecules. In this context, it comes as no surprise that the first set of metaphors adopted by synthetic biologists attempted to establish an equivalence between electronic engineering and biological systems [4,3], but without losing sight of the other domains of engineering that were still useful to the same end.

Standards for tackling the gene expression flow

From a synthetic biology perspective, there are two major aspects to contemplate in engineering living systems. One is the compositional layout, which is traditionally abstracted as layers of growing complexity from *parts* to *devices* to *systems*, with a possible intermediate stage of *modules* [3]. The second feature is the flow of information through the system, which coincides with the central dogma of molecular biology: *DNA* to *RNA* to proteins—and from there, to specific functionalities, biochemical or otherwise (Fig. 2). That the material architecture (and thus the compositional logic) of any living system is itself derived from the gene expression flow places most standardization efforts in the different phases of such a process. As every textbook would say, a coding DNA sequence can be *transcribed* to produce mRNA, which is in turn *translated* to give functional proteins. The qualitative picture is straightforward but altogether useless for robust engineering unless it is endowed of quantitative parameters, transfer functions and context-dependency data. Developing standards for these are badly needed to advance real bioengineering.

Although it is generally accepted that molecular biology was founded by physicists, it is surprising that an emphasis on rigorous metrology and higher-level abstractions has been largely absent in the corresponding literature until the onset of systems and synthetic biology. Some standards are already imposed by biology itself (e.g. the genetic code, the basic chemistry of life, or the DNA

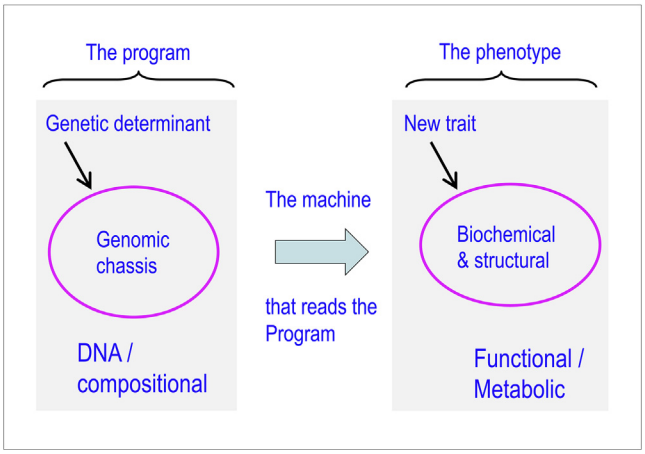

Fig. 1. The genomic chassis and its relationship with engineered genetic implants and the resulting phenotypes. By applying a computational metaphor to living systems [74], one could assimilate the DNA/genome/genetic inserts to the programme/software, and the gene expression flow machinery to the machine that reads the programme and delivers detectable functions—metabolic and others.

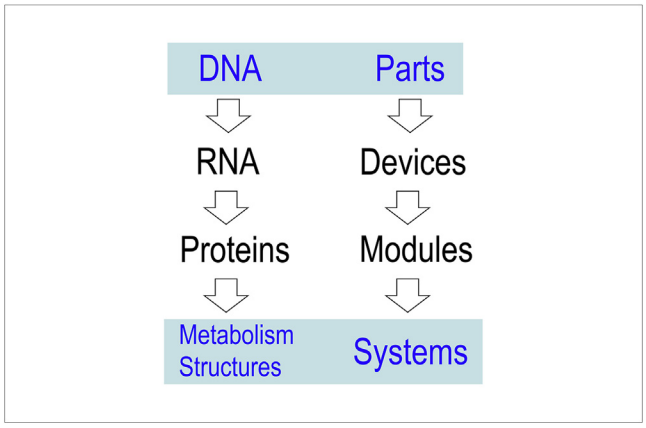

Fig. 2. The central dogma (CD) of molecular biology *vs* the abstraction hierarchy of bioengineering. Whereas the CD exposes the transfer of information through the gene expression flow, the conceptual framework of synthetic biology allows assembling complex biological systems on the basis of rationally composing parts and devices. Although the two schemes follow entirely different roadmaps, they intersect at the beginning (parts-DNA) and the end (systems-metabolism/structures).

sequences targeted by restriction enzymes). The scientific community has added very few widely accepted conventions in the field, exceptions including the EC code numbers to describe enzymatic activities, the use of β-galactosidase as a proxy to measure transcriptional activity [20], and the agreement to use specific model organisms [21,22]. Quite on the contrary, molecular biology (and the biotechnological applications derived from it) has been typically afflicted by a considerable lack of formatted methods, languages, units and rules to tackle the systems at stake. Following a 1999 DARPA report on biological circuits (https://goo.gl/R6q7eD), the first initiative to meet this issue came from Drew Endy and Tom Knight, who together with entrepreneur Randy Rettberg launched in the early 2000s a Registry of Standard Biological Parts (i.e. BioBricks: http://parts.igem.org) and the related BioBricks Foundation (http://biobricks.org) in connection with the iGEM (International Genetically Engineered Machine) competition (http://igem.org). In its original format, iGEM provided undergraduate student teams with a collection of biological parts consisting of DNA sequences encoding promoters, enzymes, reporter elements, plasmids and some combinations thereof, to design biological systems and implement them in living cells. The key angle of this initiative was that such biological parts could be reused and re-joined among them because of their formatting as DNA segments that followed a rigorous compositional standard [23]. Although the iGEM was born mostly as an educational endeavour, its tremendous success and growing internationalization has highlighted the benefits of adopting standards (even if they are very limited, as is the case in iGEM) for the sake of biological engineering. It is noteworthy that electrical and IT engineers (and not the biologists) were the first to pinpoint that the lack of quantitative standards in biology made the field something of a second-class scientific and technological exercise. Fortunately, the biological standardization momentum generated by iGEM has subsequently spread into different directions much beyond the original challenge of assembling DNA pieces. Some of these outstanding directions are briefly (and non-exhaustively) discussed below.

Physical vs functional composition of biological systems

DNA is ultimately a physical object and, as such, DNA segments can be manipulated to join other DNA segments. Apart of the classical restriction/ligation method for putting together different DNA pieces, the last few years have witnessed the booming of a large number of stratagems for assembling (i.e. physically composing) ever longer sequences [24,25]. In reality, the decisive solution to DNA assembly could be direct DNA synthesis, an endeavour that seems to become more feasible by the day—as clearly demonstrated by the recent complete chemical synthesis of a bacterial genome [26] and a yeast chromosome [27] and recent announcements to synthesize a human genome [28]. This means that although compositional rules for joining or editing DNA were at the origin of the standardization drive, this issue is basically about to be solved and such rules might not be needed in the future. But in the meantime, other fronts have opened up, as the awareness of the need for standards for bioengineering has spread, once the constructs at stake become more complex.

The immediate question in this regard is how *physical* composition becomes *functional* composition i.e. whether parts can be reused while maintaining their original properties and associated parameters [2]. The experience of the biological and biotechnological communities indicates that assembly of DNA parts often results in genetic devices that qualitatively may function as expected but quantitatively most often do not [29,30]. Genomic and biochemical context sensitivity (including physical location of the genes or the products in given locations of the 3D structure of the cell) and environmental conditions may altogether change the functioning of the parts and devices of interest [31]. In addition, designed biological systems often develop emergent properties in which the readout of the pursued phenotype may be more or less than the mere sum of its parts. This is often influenced by the small molecules that abound in any biological milieu. Last but not least, biological systems are subject to Darwinian evolution, which seems to quickly erase or silence human-made changes that cause a decrease of fitness. It is true that one can agree on very specific conditions that enable inter-laboratory reproducibility studies [32], but the same tests highlight how context-dependent biological components are and how easily they may vary, even with anecdotal environmental changes. The ultimate way out from this situation relies on having more fundamental knowledge on the rules that govern the appearance of distinct functionalities in extant biological systems through the gene flow DNA → protein in time and space—an issue that has received considerable attention in recent times [33]. But what to do in the meantime? Still for a few years, improved vectors and DNA assembly strategies that mitigate the problem of physical *vs.* functional composition will be necessary, in particular for engineering or streamlining the genomic complement of non-model bacteria (Table 2), for which less fundamental knowledge is available. One contribution in this direction was the launch in 2013 of the Standard European Vector Architecture (SEVA; http://seva.cnb.csic.es), a repository of formatted molecular tools for de-constructing and re-constructing complex prokaryotic phenotypes beyond *Escherichia coli* [34]. The SEVA is helping at this time to fill the phenomenal gap between the existing tool of DNA synthesis and the actual engineering of predictable and efficacious bacteria. Yet, although this gap is bound to rapidly narrow, the question still remains of how to convert the physical composition of DNA segments encoding genes and signals into a predictable and stable performance of the cognate bio-engineered live objects.

Reining in context sensitivity

With the aim of easing biological engineering here and now, synthetic biology proposes three avenues to handle the recurrent problem of qualitative and quantitative variations in the performance of genetic devices as they move from one context (genomic, physiological, environmental) to the other. One is the *debugging* of

extant biological systems to eliminate unnecessary complexity and thus make its layout and eventual deployment more predictable. This line of action includes not only the minimization/streamlining of genome size in order to have a more reliable chassis, but also, the decrease of regulatory complexity and fixing of otherwise variable steps in the gene expression flow [35,36]. A second option is the *orthogonalization* (mitigation of connectivity) of the engineered devices with respect to the biological host for the sake of increasing predictability [37]. This encompasses many different possibilities, ranging from alternative genetic codes and adoption of non-standard phage polymerases (e.g. from the T7 RNA phage) to physical separation of the artificial process from the rest of the cellular milieu [38]. Finally, the last few years have witnessed the development of alternative biochemistries [39] that both enlarge the molecular diversity of living systems and enable their engineering with human-imposed rules that escape the limitations of conventional biology [40–42].

However, since complete context-independency is virtually impossible, different propositions have been considered to (at least) manage it in biological systems. One interesting conceptual development involves what has been called *retroactivity* (a biological counterpart of electrical impedance [43]) that rigorously describes the mutual influence that a genetically engineered device or circuit may have on the biochemical and genomic chassis of the host—and *vice versa*, as well as the interplay between different devices that may coexist in the same host (Fig. 3). Among others, Del Vecchio's group [43] have shown that such influences may be controlled through some regulatory strategies to make each of the modules at stake independent from the others. One could therefore quantify the degree of context-sensitivity of parts and devices with a sort of *orthogonality index* and then inspect how much this could be artificially modified through additional genetic circuitry.

Metrology

Besides the challenge of standardizing assembly rules, and quite intertwined with it, the second big question of bio-engineering deals with measuring accurately biological activities. It is true that biological systems cannot be automatically equated with man-made artefacts. But, as explained above, the adoption of formalisms stemming from electrical and industrial engineering has been extraordinarily useful for the development of the field.

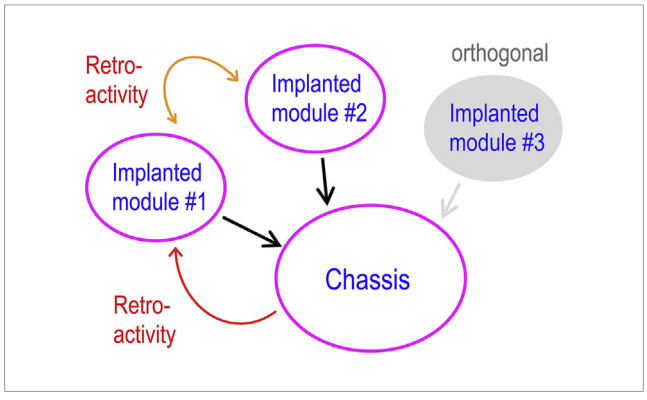

Fig. 3. Formalizing context-dependency for the performance of engineered genetic constructs. The pre-existing physiological and metabolic host (*chassis*) of synthetic devices and the corresponding constructs may mutually compete for cell resources (e.g. ribosomes, energy currrency, metabolic building blocks, *etc*). This creates a mutual perturbation called *retroactivity* [43]. In the best-case scenario, the implanted genetic modules can be made orthogonal and have little or no influence with the chassis and with other engineered devices.

While the compositional challenge of creating multi-scale biological complexity as a progression from parts to devices to modules to systems is well defined (see above), the establishment of standards for describing, measuring and rewiring key biological functionalities (as well as suitable platforms and languages for data exchange) is still a bottleneck. What is needed is the development of a new type of technologies that we could call *in vivo biomolecular metrology*. This is not only about proposing unequivocal units to describe the activity at stake, but also to figure out objects of reference for calibration so as to enable the coordination of measurements across distant locations and over time. Given that the gene expression flow rules the functioning of any biological system, it does not come as a surprise that first steps to develop a robust biological metrology start with addressing transcription and translation.

The idea of having a universal measure for transcriptional activity of given promoters was already present at the foundation of synthetic biology as a biological counterpart of electric current. The term PoPs (i.e. polymerase per second) was coined to describe the number of times RNA polymerases pass by a promoter sequence to originate a productive transcript ([44] and Fig. 4). Although transcription initiation and the quality of the resulting mRNA are in themselves quite complex and densely regulated biological events, it is possible to make a first approximation to gene expression activity by adopting such PoPs units. The next obvious question is how to measure them. Until recently, such calculations were indirect, but Steve Busby's laboratory recently developed a method to quantify physically such a parameter [45]. Although the procedures for this are fastidious and time-consuming (and still context-dependency notwithstanding, see above), having a set of well-calibrated promoters [46,35,47] in terms of their actual PoPs could be a phenomenal step for biological metrology—nor unlike the definition of amperes in

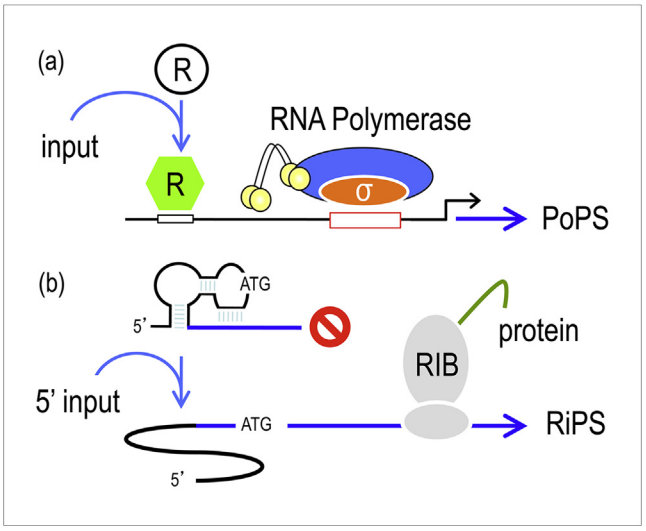

Fig. 4. The core steps of the gene expression flow. (a) Transcription. This is the most critical and controlled step in extant biological systems. In many cases (as sketched in the figure) the promoter is regulated by transcriptional factors that deliver a physical or chemical input to the promoter for generating a cognate output transcript. The number of times that RNA polymerase passes productively through the promoter sequence per second to generate a full-length transcript is called the PoPs, an absolute measure of transcriptional activity. (b) Translation. Once formed mRNA is read by a ribosome, but this may or may not be efficient depending on the availability of the 5′ region containing the leading ATG codon (a process itself dependent on possible environmental inputs). Similarly to PoPs, RiPs is number of times that the ribosome passes through a given mRNA to generate a full-length protein. Calculations on the overall outcome of gene expression thus involves both PoPs and RiPs. Figure partially redrawn from [20].

electricity. This could also pave the way for defining biological counterparts of ohms (e.g anything that impedes the progress of RNA polymerase through one promoter to the next one) and volts (e.g. inherent promoter strength). But all this still requires considerable fundamental and technological research that supports the introduction of such new concepts and parameters in biology.

The second step in the gene expression flow is translation, which could also be abstracted and parameterized as RiPs (ribosome per second), i.e. the number of ribosomes that pass productively through an mRNA sequence to deliver a full-length protein ([48]; Fig. 3). Although the abstract concept is clear, the mechanisms involved in the process are extremely intricate, in particular the control of mRNA stability and the possible targeting of mRNAs to different cells sites. Ribosome profiling [49] could help a lot to determine such RiPs parameters, but development of simpler techniques to the same end could be envisioned, with the same possible dividends as discussed for PoPs above.

Modest as they may look in a first sight, a sound definition of PoPs, RiPs units and adequate references and technologies to measure them could make a large difference to our ability to design living objects. Obviously, such metrology standards could then expand into many other biological activities amenable of rational engineering. It is not a trivial challenge, however, that these questions are not particularly *exciting* from a purely scientific point of view, but extremely *important* for the sake of converting fundamental biological knowledge into transformative real-world applications. As argued below, such efforts could thus be considered *pre-normative research* (i.e. knowledge that is necessary to generate before top-down promulgation of a given rule) and should therefore largely dwell in the public domain and under public funding rather than just left to scientific curiosity or private interests [50].

Languages for engineering biology

A third standardization front deals with languages—both for [a] description and exchange of biological data and phenomena and [b] programming cells with new capabilities. The first aspect has already received a considerable attention in the realm of systems biology and various propositions on the matter have been entertained over the years. One of the simplest involves logic gates: regulatory networks possess a large number of control modules that formally implement many of the operations that are typical of digital, Boolean circuits [51]. As the corresponding biological transactions adopt somewhat continuous values, the 0/1 states are generally agreed to reflect low/high states for the input status and off/on for output promoter activity. Logic gates based on promoters and transcriptional factors provide an attractive and simple (while also scalable) framework for both describing and designing artificial biological circuits [52,53], as a virtually unlimited diversity of schemes can be produced just by combining a relatively small number of modules (Fig. 5). A far more sophisticated approach is the so-called Systems Biology Markup Language (SBML, http://sbml.org), which defines itself as a machine-readable format for representing models and oriented towards describing systems where biological entities change over time e.g. regulatory networks and biochemical reactions [54–56]. SBML claims to be a framework suitable for representing models commonly found in research on a number of biological topics, including cell signalling pathways, metabolic pathways, biochemical reactions and gene regulation. Interestingly, SBML does *not* pursue a universal language, but a common intermediate format—a sort of *lingua franca*—enabling communication between different models and different communities. This is an interesting aspect which contrasts with other standardization efforts. While metrology aims to define permanent, universal units to describe and

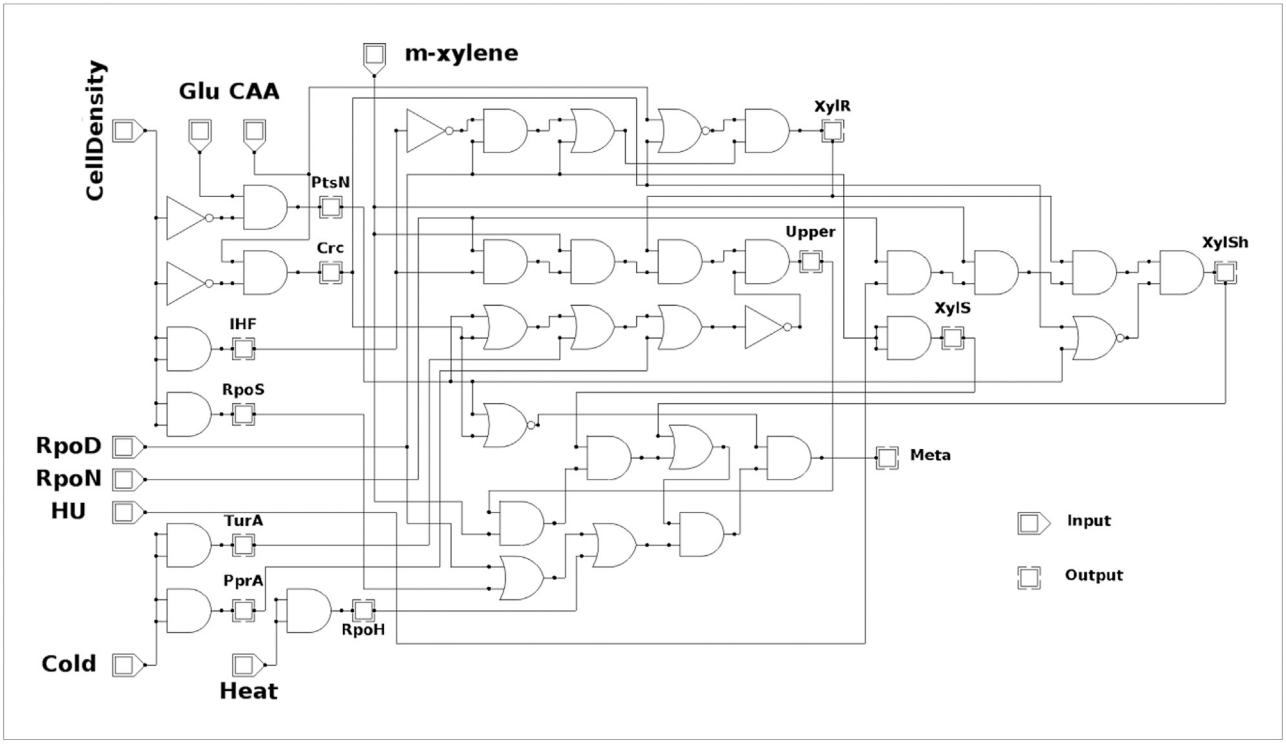

Fig. 5. Boolean logic gate representation of complex biological networks. Logic gates afford representation and modelling of typical regulatory actions in a control network even in the absence of detailed parameters. The image shows the relational logic structure (the so-called *logicome* [53] of all known molecular actors involved in expression of the TOL pathway of the soil bacterium *Pseudomonas putida* mt-2 for catabolism of the environmental pollutant *m*-xylene. The circuit shown allows the bacterium to compute a large number of internal and external cues for deciding expression or not of the corresponding metabolic route (figure kindly provided by Rafael Silva-Rocha).

measure biological activities in a lasting fashion, languages may by definition evolve, coexist and need mutual conversion. The emphasis in this field thus seems to be placed more in ways of standardizing translation between them than on generating one sole standardized grammar and lexicon.

A more recent (and somewhat more assertive) proposition is the Synthetic Biology Open Language (SBOL http://sbolstandard.org) that focuses on genetic designs through a standardized vocabulary of schematic glyphs as well as a standardized digital format [57–59]. One major appeal of SBOL is the specification of unequivocal rules to visually represent either natural or engineered genetic circuits, which can then be enriched, also following strict rules, with experimental and computational data. This allows detailed descriptions not only of specific circuits, but also of entire workflows of biological engineering [60]. The SBOL format is rapidly being adopted by a large number of communities (including journals) and it may end up being the preferred instrument of communication between biological systems, human users, computational resources and even robotic platforms for remote experimentation. In this respect, it is noteworthy that standardized programming languages are also being settled for describing and implementing experimental *wet* protocols in biology. Take for example current platforms to outsource scientific experiments to the best bidder (e.g. see: https://goo.gl/0Wfn6l or http://www.transcriptic.com). Once researchers get used to the fact that scientific work in the laboratory is treated in principle no differently to the production of any other goods or services, why not formalize and further standardise the process? The BioBlocks platform has been developed for just that, as it allows one to automate the execution of experiment in a fashion that can be specified, saved, modified and shared between multiple users in an easy manner [61]. BioBlocks is open-source and can be customized to execute protocols on local robotic platforms or remotely i.e. in the cloud (see: http://vps159.cesvima.upm.es/software/Bioblocks). It aims to serve as a *de facto* open standard for programming protocols in biology. Development of standardized languages like BioBlocks or Antha (https://www.antha-lang.org), that can ultimately be understood by a robot or artificial intelligence (AI), can be indeed revolutionary: one given genetic construct may be designed by humans or AI in one place, simulated in another location and finally tested experimentally in yet another robotic or microfluidic platform somewhere else. A more modest proposition, but still extraordinarily useful when implemented, is the standardization of protocols and procedures done in different laboratories to improve reproducibility and interoperability, such as proposed *inter alia* by https://www.protocols.io. The consequences of such scenarios go well along those of the 4th Industrial Revolution, where delocalization, atomization, commodification, automation and production-on-demand [10] may well replace the craftsman type of bio-laboratories and biotechnological setups that we are still used to seeing today.

There is still another type of standard languages: those that allow programming cells to sense signals, run logic operations and make decisions in a way not unlike electronic devices do. A phenomenal step to this end has been the recent development of CELLO (http://cellocad.org/), a platform to design genetic circuits that perform given computational operations and which the user can connect to sensors (the inputs) and cellular functions (the outputs). The user simply provides the DNA sequences for the input promoters (the sensors), data for their ON/OFF signal strengths (in standardized units) and then connects the output promoter to the desired cellular function [62]. The system then returns a solution (including a DNA sequence proposition) for such a circuit. Since the basic unit of computation for complex control systems is the NOR gate, CELLO plays with a large number of promoter/repressor combinations that deliver such logic and in a format that can be combined according to users' needs. Programming languages of this sort are likely to be refined and further developed to make biological engineering much closer to *bona fide* engineering, including computer engineering [62].

Storing and managing information

While, as argued above, the three core fronts of the biological standardization challenge involve functional composition, metrology and language, the story would not be complete without addressing the issue of data management. The existing Repository of Standard Biological Parts (http://parts.igem.org) has already a good number of its listed items associated with datasheets similar to those widely used in engineering [63]. Such datasheets provide a template for producing many standardized genetically encoded objects. However, the repository is not professionally curated (i.e. data largely comes from iGEM teams as a collateral product of their student work) and the reliability of the contents is based more on the popularity of the genetic devices than on rigorous and peer-reviewed experiments. But despite its shortcomings, this database is still a major resource of information for easing biological engineering. A more professional platform in the same realm is that developed at Imperial College London under the denomination DICOM-SB (http://synbis.bg.ic.ac.uk/dicomsb/), which is inspired by the highly successful Digital Imaging and Communications in Medicine (DICOM) standard. The system captures all the data, metadata, and protocol information associated with biopart characterization experiments [64]. The platform can accumulate and process large amounts of data and includes services orientated towards interoperability and automatic exchange of information between different modalities and repositories i.e. it has been designed to be compatible with and complementary to other standards in synthetic biology, including SBOL (see above). The DICOM-SB data model forms part of the web-based information system, SynBIS. It is possible that other platforms will develop in the future to serve specific needs as the new biotechnology expands and diversifies. Moreover, as discussed for languages, it is possible also that standards for interoperability between databases and associated resources will become more useful than the standards followed by each of the separate platforms. An overarching initiative in this respect is ELIXIR (http://www.elixir-europe.org), a Europe-based platform for managing and safeguarding the increasing volume of data being generated by publicly funded research. The infrastructure coordinates, integrates and sustains bioinformatics resources across its member states and enables users in academia and industry to access vital services for their research. A considerable focus is placed on interoperability: ELIXIR encourages the life science community to adopt standardized file formats, metadata, vocabularies and identifiers. This helps both humans and computer software to discover, integrate and analyse (big) data. This objective is brought about by an interoperability platform, a group of experts drawn from across Europe, although it has a global perspective. Other resources for data reposition and interoperability (e.g. FAIRDOM https://fairdom.org/) are becoming popular as well—as long as funding bodies request beneficiaries to use them obligatorily.

Enabling standards for increased biosafety and easier risk assessment

Since the early days of genetic engineering, biosafety concerns have been brought up and discussed [11,65]. The Asilomar meeting in 1975 can be seen as the starting point for the implementation of a safety protocol and an attempt to quantify risks into different categories (e.g. biosafety levels 1–4). While initial fears mostly turned out to be unsubstantiated, a number of guidelines and regulations can be traced back to these early precautionary visions

of the power and scope of genetic engineering [66]. But although safety levels exist, and institutional biosafety boards oversee the work in the laboratories and national and international laws regulate the use of genetically modified organisms, there is still concern that science and engineering could design and engineer novel life forms with a serious risk to human health or the environment. Contemporary preoccupations about synthetic biology, CRISPR/Cas9, gene drives etc., and the way they could potentially cause harm appear not too dissimilar from what James Danielli wrote in 1972 in his landmark article *Artificial Synthesis of New Life Forms* [67]. In other words, 40 years into genetic engineering, it seems we have still not sufficiently dealt with the issues of biosafety and risk assessment, especially in the light of new methods and technologies, and of course standardization efforts to make biology easier to engineer. We believe it would be an act of ignorance to disregard these biosafety concerns when discussing standardization needs for the KBBE. Instead, we argue that improved standards in biosafety and risk assessment are in fact a key requirement for the success and sustainability of the bioeconomy.

While we acknowledge that the present risk assessment methodologies are appropriate for assessing potential risks of contemporary synthetic biology activities and products, we agree with a recent opinion by the European Commission's Scientific Committee for Emerging and Newly Identified Health Risks[1] (SCENIHR 2015) concerning research recommendations for risk assessment in synthetic biology. The SCENIHR suggested several improvements to ensure continued safety protection proportionate to risk, while at the same time enabling scientific, technological and socio-economic advances in the KBBE. The SCENIHR opinion lists 5 major starting points for improvement: [i] support the characterisation of the function of biological parts and the development of computational tools to predict emergent properties of synthetic biology organisms, [ii] streamline and *standardise* the methods for submitting genetic modification data and genetic parts information to risk assessors, [iii] encourage the use of GMOs with a proven safety record as acceptable comparators for risk assessment, [iv] aim to ensure that risk assessment methods advance in parallel with synthetic biology advances, and [v] support the sharing of relevant information about specific parts, devices and systems with risk assessors. According to their recommendations, an important aspect seems to be the ability to precisely describe the engineering and share it in a highly structured way with those in charge of the risk assessment. Standardisation should thus also enable the risk assessment process to be comprehensive, precise and adequate and be carried out in a time efficient manner without sacrificing quality and certainty. The recommendations of SCENIHR were made to cover a period defined as the next 10 years (beyond which any scenario might rather qualify as science fiction in this field). For this period SCENIHR was concerned that a lack in the support of standardization on how to obtain and share risk assessment data could lead to an upcoming bottleneck for real world applications of synthetic biology.

In a scenario where risk assessment would rely on contemporary methods alone, and where synthetic biology is able to design and produce novel life forms that differ from wild types in a much deeper and substantial way, risk assessors are going to face considerable difficulties in trying to assess the cases in front of them, as they will take too long, will be massively understaffed and have difficulties understanding the level of change and potential impact of the engineered organisms. Such a situation of *structural incompetence* could lead to two, undesirable outcomes. In the first *permissive* scenario, quality is sacrificed over quality, leading to too many (and also some harmful) applications that would pass the assessment and later on cause harm in one way or the other. In the second *restrictive* scenario, quality is maintained over quantity so that too many (and safe) applications would not be granted that would have contributed to a flourishing bioeconomy and society. Obviously both scenarios are suboptimal and a hindrance for the KBBE, which means there is no alternative to properly addressing and supporting the development and use of standards for biosafety purposes.

SCENIHR also recommended the support of research and development of novel types of biocontainment, (sometimes called e.g. genetic firewall, intrinsic or semantic biocontainment) to add an additional level of containment and safety for real world applications such as human medical useindustrial biotechnology or large scale agri- or aquacultural deployment. As described in [68–70,66] these types of containment have the potential to increase the control over horizontal gene flow and environmental persistence by altering fundamental characteristics of living systems, such as the biochemical composition of key biomolecules or even the genetic code. While in recent years a number of high impact publications [71,72] have demonstrated the enormous potential that lies in these xenobiological or semi-synthetic organisms, there are hardly any metrics available to keep pace with the tremendous improvements. For example, almost the only metric currently available for auxotrophic systems is the evaluation of the escape frequency. In a recent study about biocontainment of GMOs containing synthetic protein design, [71] stated: '*Our results demonstrate that mutational escape frequency under laboratory growth conditions is a necessary but insufficient metric to evaluate biocontainment strategies*'. Unfortunately, this metric has (at least) two major shortcomings: [i] The detection limit to assess the escape frequency is about 10^{-11}. In order to be considered for a release into the environment, a physically contained industrial fermenter or even into a human patient, the escape frequency will have to be significantly lower than that. Furthermore, [ii] there are no standards in terms of the media to test the escape frequencies in different environmental contexts. [72] for example evaluated their synthetic auxotrophic strains on blood agar and soil extracts. So far there is no agreement on the set of media that needs to be used as a standard in risk assessment. Developing sufficient metrics to evaluate a genetic firewall and definition of media is indispensable in order to advance the construction of intrinsic biocontainment systems. Once bioengineering becomes a widespread exercise, the field will also have to incorporate benchmarks and best practices available in what can be called *biosafety engineering* [70].

Status quo *and outlook*

Despite the considerable benefits that adopting biological standards on the diverse fronts discussed above could bring to the biotechnology of the future, virtually all initiatives thus far in that respect have been bottom-up. If one inspects the recent compilation by Schreiber et al. [73] on past and ongoing standardization initiatives in systems and synthetic biology, it becomes clear that this has been mostly a self-driven community matter, with a very limited intervention of regulatory or standardization authorities thus far. The earlier ideas on standardization associated with iGEM and the Repository of Biological parts, were followed and expanded by the BioBricks Foundation (http://biobricks.org) and the iGEM Foundation (http://igem.org). In a phenomenal push to the field, the National Science Foundation supported, during the period 2006–2016, the so-called SYNBERC (Synthetic Biology Engineering Research Center https://www.synberc.org), a joint endeavour of 5 top US universities with the objective of laying the conceptual and material foundations for synthetic biology and bioengineering.

[1] The co-author was part of the Working Group responsible for the final opinion.

One of the working groups dealt specifically with standards for measurement and characterization of biological parts and produced useful datasheets that have fed excellent information to various bioengineering platforms. SYNBERC has since been followed by the Engineering Biology Research Consortium (EBRC https://www.ebrc.org/), which includes a much larger number of academic partners and puts a considerable emphasis on industrial leadership and involvement of public stakeholders. However, the first time that an official US authority moved from being a supportive observer/funder to become an active player in the field of biological standards occurred in 2015 with the creation of a *Synthetic Biology Standards Consortium* fostered by the *National Institute of Standards and Technology* (NIST https://goo.gl/gpwN3C). One of the most active pursuits under this umbrella is the programme called *The Joint Initiative for Metrology in Biology* (JIMB http://jimb.stanford.edu), an alliance of academic and industrial entrepreneurs linked to Stanford University with experts from NIST to cover a range of standardization issues aimed at powering the bioeconomy. One of JIMB's major activities is the Synthetic Biology Standards Consortium (SBSC) (http://jimb.stanford.edu/sbsc), a private public partnership to '*collectively build the metrology infrastructure to support a fully integrated, global synthetic biology enterprise*'.

Regrettably, nothing remotely similar to the initiatives mentioned above has happened at the EU level, although some countries (e.g. the UK) have had some national-level programmes to tackle the standardization issue. For instance, during the period 2008–2011 under the research initiative *Networks in Synthetic Biology,* the Biotechnology and Biological Sciences Research Council (BBSRC) co-sponsored a British consortium on *Standards for the Design and Engineering of Modular Biological Devices* (https://goo.gl/DicwEF). Echoing many of the ideas raised before by SYNBERC, this programme addressed questions on measurement of biological parts, building computer simulations on their performance and also considered other aspects of parts-based synthetic biology, such as intellectual property rights. More recently, the so-called FLOWERS Consortium (http://www.synbiuk.org/) of five UK universities was set up to make synthetic biology a well-characterized instrument for industrial applications. It is worthy of note that FLOWERS places a considerable emphasis on setting information and experimental infrastructures able to develop standards for CAD, models, chassis, parts and device characterisation and DNA assembly—all issues that belong to the core of any serious standardization agenda.

Unfortunately, official interest in biological standards (other than databases, see above) did not spread significantly in Europe beyond UK borders. The COST Action called CHARME (http://www.cost-charme.eu) with participants from 26 European countries and expected to operate for the period 2016–2020 was recently set up to inspect the need for pooling, networking and harmonising the various activities on standards in the EU. The starting point for this action is the fragmentation and disconnection of ongoing initiatives and institutions to develop and implement standards in the life sciences, such as those launched by the International Organisation for Standardisation (ISO) or the German Institute for Standardisation (DIN). CHARME, however, only focuses on languages, software, dissemination, legal aspects and networking of possible stakeholders, without addressing the outstanding scientific and technical questions that still need to be tackled (see above) to convert biotechnology into an authentic engineering discipline. In contrast, other European platforms (e.g. The European Forum for Industrial Biotechnology and the Bioeconomy http://www.efibforum.com) have precisely pinpointed the strategic importance of standards based on sound science to drive the transition towards a circular BioEconomy in Europe (https://goo.gl/oNBWy6).

One key European actor in the field of standards is CEN—CENELEC (http://www.cencenelec.eu), which merges the three officially recognized European standardization organizations: the European Committee for Standardization (CEN), the European Committee for Electrotechnical Standardization (CENELEC) and the European Telecommunications Standards Institute (ETSI). CEN and CENELEC bring together the national standards agencies of 34 European countries and include business federations, commercial and consumer organizations, environmental groups and other societal stakeholders–reaching out to more than 60,000 professionals. It is alarming that in their appraisal of standardization-related EC-funded programmes in the ongoing H2020 Framework, the topic of Standards for Synthetic Biology and Bioengineering is nowhere to be seen (https://goo.gl/FpZBml). Even the one significant project on biological standards funded by the EC under the 7th Framework Programme (ST-FLOW, https://goo.gl/OL2kOV) escaped the notice of CENELEC in success stories on standardization in Europe (https://goo.gl/1I3olz). The conspicuous lack of interest of EC-level bodies on biological standards is also shown by the absence of the two reports on the matter that originated in respective meetings run under the aegis of the *Synthetic Biology Working Group* of the *EC-US Task Force in Biotechnology* (https://goo.gl/HSVdhO) in 2010 and 2012 and the recommendations of the US and EU stakeholder meeting of scientists, industry players and representatives of the major funding agencies co-sponsored by the EC and the NSF in 2015 (https://goo.gl/M0o8k9). This meeting was participated in by the NIST, which shortly afterwards launched in the US the above mentioned *Synthetic Biology Standards Consortium* —with no matching initiative whatsoever on the European side. Important but somewhat stand-alone initiatives do happen occasionally. For instance, the SPIDIA Project (www.spidia.eu) on standardization and improvement of pre-analytical procedures for *in vitro* diagnostics. This Project fostered the first 9 CEN Technical Specifications (CEN/TS) for pre-analytical workflows in Europe. But, valuable as they are, such projects are not framed on a long-term policy on the matter.

The lack of involvement of continental Europe in the science and technology around biological standards that is proceeding in other parts of the world may have serious consequences for the development of the KBBE in the EU and can compromise the leading position in the 4th industrial revolution that Europe aims to have. It is also of concern that the International Organization for Standardization (ISO, http://www.iso.org), the world-wide authority in the field with competence on virtually any type of industrial, medical or environmental activity and with >20000 standards promulgated thus far, has paid little or no attention to bioengineering and its specific standardization challenges. Given that (as substantiated above) standards make the difference between biotechnological trial-and-error and engineering in earnest, it is to be hoped that sooner or later this endeavour will be widely recognized as one of the drivers of our future industry and economy.

Conclusion

Without trying to be exhaustive, the sections above illustrate how, due to systems and synthetic biology, modern biotechnology is becoming more and more comparable to authentic (not just metaphorical) engineering. As is the case with engineering, adoption of standards makes a difference in terms of the scalability, reproducibility and predictability of the endeavour. In fact, there are a few historical lessons that can guide the next steps and identify bottlenecks that need to be overcome for the KBBE to deliver its promise. The laws of electricity, its parameters, units, devices and their transformative applications for society and economy for a little more than a century were preceded by a huge

fundamental effort to understand physical phenomena. How does this relate to KBBE in the frame of the 4th industrial revolution? Evidence clearly indicates that those who develop the foundations of a new scientific and technical discipline, and its associated standards, become the longstanding leaders in the field. There is thus a need to bring not only fundamental biology at large, but specifically research on standards to the agenda of KBBE. As discussed above, some standards can be implemented immediately (such as the physical composition of DNA sequences, tools for genome editing, languages to encode workflows), while others look like low-hanging fruits (biological metrology, operative systems for bio-programming cells) and finally others will require a considerable effort such as understanding the rules for functional composition of biological activities.

Sooner or later, biological standards will also become a matter of high-level agreement for their promulgation and eventual enforcement. Despite some very visible, impressive achievements of the last decade, the type of advanced biotechnology that we envision in this article is still in much need of establishing platforms of reference (e.g. genomic chasses, molecular vectors, design and assembly tools, operative systems, characterization of biological modules, fail-fast schemes, *etc.*) before the field can have a transformative impact on industry. This also requires the flow of public research funds into the field, which is not without successful precedents. Public funding played a major role in the birth of the computer revolution and has supported the physical infrastructure needed for frontline research and the education of professionals who now feed the IT industry. Synthetic biology-based biotechnology is not just a technical change: it has the potential to transform our societies by creating new types of jobs and industries e.g., from *Si Valleys* to *CHNOPS Valleys* (http://www.eoht.info/page/CHNOPS). In the last scenario, the chemical elements that shape living systems could co-exist and even replace in many cases the type of industry that we are familiar with. We also believe that the analyses above can be instrumental for ensuring that a new type of biotechnology moves quickly and responsibly from laboratory experiments to large-scale processes that enable a true KBBE. Simultaneously, we argue that early involvement of the public, amateur biologists and other stakeholders will help steer the direction of technology in socially acceptable and responsible ways, rather than simply avoiding a repeat of the European experience with GM crops. We need to train a next generation of biosafety leaders and enable proper government oversight and development of tools for establishing and sustaining trust across borders of secrecy, and in taking ownership of a public strategy to enable future biosecurity.

In this desirable pursuit of a better future, we note that the EU as a whole and continental Europe in particular is not capitalizing sufficiently on its scientific and technical community to ensure a leading position in the pathway towards a KBBE (see a discussion on this in https://goo.gl/h9ptfX). In our view, the challenge is not so much the lack of bottom-up interest, but the dearth of responses of European funding, policy and regulatory agencies to the unprecedented and somewhat unsettling challenge of developing standards for living objects. The involvement of the European Committee for Standardization (http://www.cen.eu, see above) seems to be urgent at this point, as it has already in place the channels to propose new standards for a variety of fields that have been previously approved in dedicated workshops (https://goo.gl/cncTJA, https://goo.gl/faswsh). This has to be supported by a sustained backing of national and Europe-wide funding agencies, as well as the industrial sector, to tackle fundamental biological questions that still need to be settled as described earlier in this article.

The Metre Convention signed in 1875 created the International Bureau of Weights and Measures (http://www.bipm.org). This organization has had since had world-wide authority in matters of metrology, standards of ever increasing accuracy, range and diversity—and equivalences between diverse measurement standards. If European leadership is serious about KBBE, then the standardization agenda needs to be expanded soon towards the area of biotechnology. Otherwise Europe will have to follow the example of others.

Conflict of interest

Authors declare no conflicts of interest.

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