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Engineering and ethical perspectives in synthetic biology

Rigorous, robust and predictable designs, public engagement and a modern ethical framework are vital to the continued success of synthetic biology.

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Synthetic biology has emerged as an exciting and promising new research field, garnering significant attention from both the scientific community and the general public. This interest results from a variety of striking features: synthetic biology is a truly interdisciplinary field that engages biologists, mathematicians, physicists and engineers; its research focus is applied; and it has enormous potential to harness the power of biology to provide scientific and engineering solutions to a wide range of problems and challenges that plague humanity. However, the power of synthetic biology to engineer organisms with custom-made functionality requires that researchers and society use this power safely and responsibly, in particular when it comes to releasing organisms into the environment. This creates new challenges for both the design of such organisms and the regulatory process governing their creation and use.

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As synthetic biology is being defined and developed by researchers spanning several fields, it is hardly surprising that a unified definition of synthetic biology is lacking. For the purposes of this paper, we define synthetic biology as 'the endeavour to design new, or modify existing, organisms to produce biological systems with new or enhanced functionality according to quantifiable design criteria', because it explicitly requires that the synthetic system can be evaluated against a quantifiable design objective as is done in traditional engineering.

The origins of synthetic biology go back to 1979, when the Nobel Prize-winning chemist Har Gobind Khorana synthesized a 207 base-pair DNA sequence [1]. Since that breakthrough, the size and complexity of synthetic DNA molecules has rapidly increased, culminating in 2010 with the successful synthesis of the 1.08 Mbp *Mycoplasma mycoides* genome, which is capable of self-replication [2]. This breakthrough prompted US President Barack Obama to commission a report on the safety implications of synthetic biology. In December 2010, the Presidential Commission for the Study of Bioethical Issues (PCSBI) reported to the White House and "found no reason to endorse further federal regulations or a moratorium to work in this field at this time" [3]. The report recommended that scientists should regulate themselves and pointed out the need for "scientific, religious and civic engagement with the public". Such recommendations, especially in regard to public engagement, had already been made by the main UK funding bodies: the Engineering and Physical Sciences Research Council (EPSRC), the Biotechnology and Biological Sciences Research Council (BBSRC), the Economic and Social Research Council (ESRC) and the Arts and Humanities Research Council (AHRC).

The rationale for such studies and reports is the enormous potential of synthetic biology to develop new biological organisms capable of carrying out functions useful to industry and society, such as waste degradation, water toxicity detection, biofuel production, drug development and so on. Many of the applications of synthetic biology ultimately involve the release of synthetic organisms into the environment. In addition to raising social and ethical issues, this creates major scientific and engineering challenges, in particular the need to design robust and predictable synthetic organisms. Thus, until we are capable of constructing synthetic organisms that meet strictly defined design criteria—in which future behaviour is predictable in the presence of intrinsic and extrinsic noise, uncertainty and evolutionary action—scientists and society should exercise caution before synthetic organisms are allowed to leave the confines of the laboratory. Robustness requirements, such as these, need to be incorporated into the synthetic biology research cycle as is common practice in other engineering disciplines (Fig 1).

Contrary to typical engineering designs, biology exploits unavoidable variability to ensure robustness and adaptation to changing environments

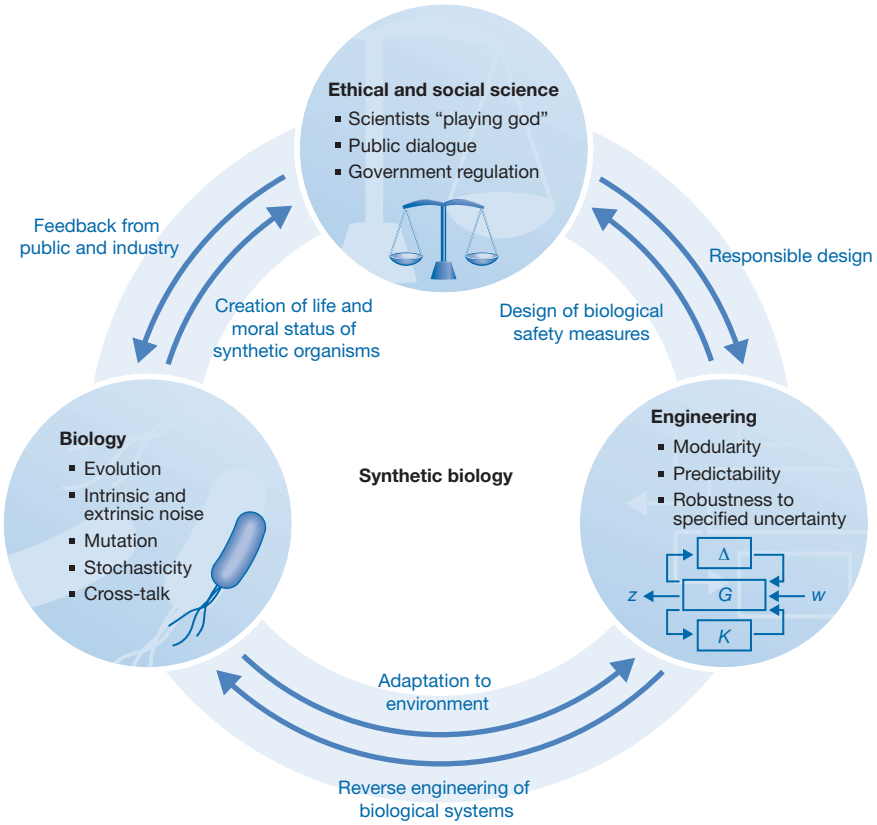

Fig 1 | Interaction between the three key subject areas in synthetic biology.

Technological systems of staggering complexity, such as digital computers, power grids and the Internet, have become such an integral part of our lives that we take them for granted. Their success owes, in large measure, to the modular design strategy that engineers use to deal with escalating levels of complexity and conflicting requirements. A large system is typically designed by using a 'top-down' approach: the problem is divided into a hierarchical set of smaller sub-problems for which it is easier to design and implement smaller subsystems using existing and well-characterized modules that solve these sub-problems. The task is complete when the overall design has been successfully tested and verified, to guarantee that the system performs according to the specifications to which it was designed. If the design is not modular and hierarchical, verification rapidly becomes intractable.

Unfortunately, it is not always clear what constitutes a module in biological systems, nor how modules can be interconnected. One way to define a modular structure in natural genetic circuits is through 'network motifs', interaction patterns that occur frequently in complex networks and that could be associated with particular functionalities. Such motifs can range in scale from localized bimolecular interactions to complete pathways, such as linear cascades or coherent and incoherent feed-forward loops. Although various algorithms have been developed to identify such motifs in protein–protein and genetic networks in *Escherichia coli* and *Saccharomyces cerevisiae* [4], the relationship between network motifs and the dynamic functionality of the whole network is still unclear. This conceptual gap needs to be resolved if we are to use natural modules systematically to redesign predictable and robust synthetic organisms. It is therefore not surprising that the first synthetic systems used simpler configurations, rather than adaptations of more complex biological networks, to achieve a particular functionality. Instead, those constructions were based on classical engineering design principles (Table 1).

Part of the success of synthetic biology to date has depended on a measure of modularity, through the creation of a standard library of parts, that enables a 'plug-and-play' framework for biological circuits. The idea is to develop a library of standardized genetic modules with specific functionality that can be combined to achieve a certain function, analogous to binary transistor logic libraries used by electrical engineers. The Massachusetts Institute of Technology (MIT) Registry of Standard Biological Parts is, at the time of writing, the most popular example (http://partsregistry.org/).

Although the 'second wave of synthetic biology' [5] now focuses on the interconnections of such modules, the issue of modularity is still open to debate [6]. The question is whether modularity is a natural property, or whether it is an abstraction imposed by engineers to simplify the design of complex systems. One school of thought asserts that abstraction should be enforced in synthetic designs to achieve scalability, predictability and robustness. The opposing view contends that dictating that all synthetic biology designs must be modular, can lead to unworkable constructs and significantly restricts the design options.

At the other end of the spectrum, engineers must make sure that their designs are 'robust' to noise and perturbations, or, put another way, can operate in the presence of uncertainty. Such robustness is achieved, often at great cost, typically through the use of feedback loops [7]. However, making a system robust to a particular set of perturbations can make it fragile to other perturbations, which results in 'robust yet fragile' behaviour [8]. If the designer is aware of this constraint, he or she can ensure that overall fragilities are rare. A commercial airliner for instance has been designed to be robust to perturbations such as cargo load variation, atmospheric changes or ageing material. Each of these perturbations occur over different but known spatial and temporal timescales. However, unanticipated microscopic damage to a few core processors on board could have catastrophic consequences.

In the context of synthetic biology, the balance between robustness and fragility is extremely important. Consider as an example the case of a synthetic biosensor to detect land mines [9]. Researchers would have to create designs and describe various biochemical reactions and the organism's interactions with the desired target. However, only a finite number of probable interactions can realistically be modelled. Furthermore, each interaction can be modelled only with respect to a few parameters,

such as temperature, pH levels, kinetic constants, evolutionary fitness and so on. Assuming the constructed organism satisfies all the design criteria and is robust to the uncertainty just described, is it reasonable to release it into the field? Clearly, the job of detecting land mines is a worthy cause; however, there are probably many other factors that the biosensor encounters that have not been modelled or accounted for, or for which the parameters considered during the modelling phase are not valid.

Simple organisms use feedback to achieve such robustness—the classic example is chemotaxis in *E. coli* [10]. Robustness applied to synthetic biology should ensure that the dynamic behaviour of the engineered organism is not sensitive to 'small' expected fluctuations in the environment in which it operates. However, one of the fundamental challenges for synthetic biology is to account for the uncertain environment that the synthetic organism might eventually inhabit. This uncertainty is caused by a variety of factors—high levels of noise, adaptation and evolution at the organism and population levels, and uncharacterized cross-talk between modules—and is fundamentally different from that typically accounted for in engineering.

A systems engineering approach to synthetic biology advocates a separation of the design into two distinct layers: the design of individual components or modules to create the desired functionality, and the design of their interconnection structure and communication mechanisms. Two key issues under debate are whether natural systems can be interpreted as hierarchically organized modules, and conversely, to what extent synthetic biologists can learn from the interconnection architecture of natural systems (Table 1).

These two questions lead naturally to the overlap between synthetic and systems biology. The latter studies the organizational structure of natural systems to derive simplified models, by using assumptions such as modularity and hierarchical layering. Indeed, there are similarities between natural and man-made complex systems in terms of their organizational structure, as illustrated in [11] by using a Lego brick analogy. However, despite these apparent organizational similarities, some of the defining properties of biological systems, such as their natural ability to evolve, raise fundamental theoretical challenges.

Table 1 | Engineering, natural and synthetic solutions for designing complex systems

Design challenge	Engineering solution	Natural organism	Synthetic biology solution
Scalability	Modularity	Motifs Modularity?	Modularity Well-characterized modules
Retroactivity/ cross-talk	Insulation and feedback	Feedback	Orthogonal design
Robustness	Feedback	Feedback	Feedback
Complexity	Hierarchical design	?	Hierarchical design
Evolution/ mutation	?	Efficiency/robustness trade-off	?

A main obstacle to designing predictable and robust biological circuits, is the requirement for exact input–output properties of modules both in isolation and when interconnected. Many of the synthetic parts in the MIT registry are poorly characterized in regard to their dynamic behaviour in isolation and under varying biological environments. This is in part caused by a lack of knowledge about the environmental variables that can affect biological behaviour. In a classical engineering set-up, a sufficient description for a module would involve a predictable input–output behaviour, characterized by parameters that can be varied to suit key properties—such as operating temperature, pressure, energy source and so on—that define the state of the environment in which the module is to operate. In biological design, many of the environmental properties that determine the behaviour of a module have not been identified, or have been identified but not modelled owing to their complexity.

Even if we assume that a module is fully characterized in isolation, there are further challenges related directly to our ability to predict the behaviour of an ensemble of interconnected modules. One such obstacle is 'retroactivity'—feedback from downstream events and elements that typically propagates information in a direction opposite to that which is normally anticipated [12]. Such effects are relevant because any useful synthetic organism is probably formed from multiple interconnected modules. From an engineering perspective, each module is designed so that retroactivity is attenuated around the desired operating point of the system. An example of retroactivity is a cascade of hydraulic tanks: when water flows from an upstream tank to a downstream tank, the water pressure in the upstream tank changes. The result is a flow of information in both directions although the input to output behaviour acts only downstream. Retroactivity in cellular biology appears when an upstream promoter experiences feedback from the activation of downstream promoters, as observed experimentally in the nitrogen assimilation control system of *E. coli* [13]. It has been shown, however, that long phosphorylation and dephosphorylation cascades can significantly attenuate retroactivity [14]. Thus, one possible solution could be to incorporate these cascades into synthetic constructs to increase the level of modularity of the designed system.

Another difficulty for designing predictable behaviour in interconnected modules is the fact that different connected modules might have varying sensitivity to the intra- and intercellular environment. Consider, for example, a synthetic organism that is designed for waste degradation [15] and assume that such an organism comprises several modules. In addition to the differing sensitivity of the connected modules to intra and intercellular environments under laboratory conditions, the variable and uncertain environment in which they are to be released will push the operation of the engineered organism to its robustness limit. With respect to waste degradation, this potential lack of robustness is a severe problem, as a population of synthetic organisms would need to be sustained to be effective. Unique to this example is the fact that once the synthetic organism—which for the sake of argument we shall say is 'robust'—has achieved its goal, it might not be desirable to continue the synthetic population. An overly robust organism might even become problematic unless we can control where the 'fragility' occurs and use this as a form of population control. By maintaining this line of thought it might be possible to specifically engineer

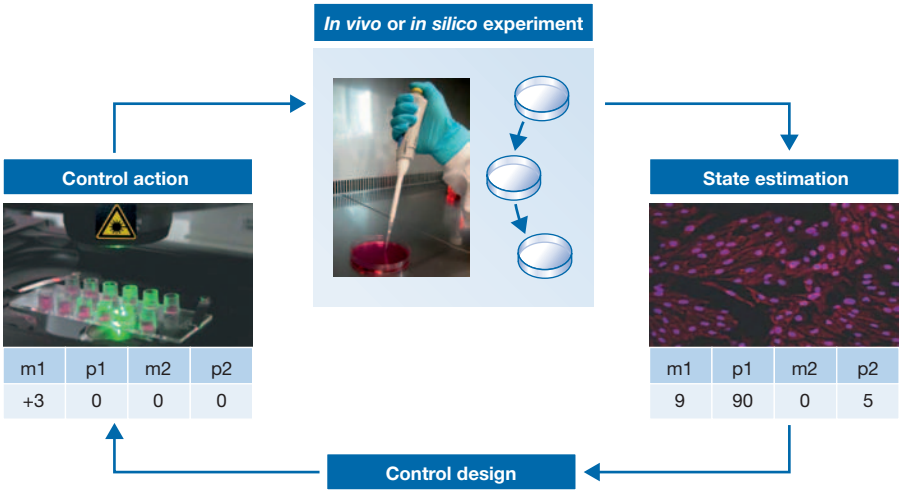

Fig 2 | Conceptual idea of incorporating a feedback signal to sustain a synthetic population.

fragility in such a manner that the synthetic organisms are 'evolutionary losers'. When the synthetic organisms are released into the open environment they will be outcompeted by their natural counterpart and eventually die off.

Another solution to address intra- and intercellular sensitivity is to design 'orthogonal' modules that do not interfere with each other. Recent work [16] created a synthetic pathway in *E. coli* on the basis of the idea of transcription and translation mechanisms that are orthogonal to those naturally present in the bacterial host cell.

One of the most challenging aspects of synthetic biology is the engineering of evolution

A theoretical approach for designing a controllable synthetic circuit has been proposed [17,18] and could be applied in similar situations. The principal idea is to incorporate an outer feedback loop around the synthetic construct, such that the organism cannot be sustained without an external control signal: a given nutrient or a light source. This type of approach guarantees that the engineered organism cannot survive unaided in the environment and will eventually die off when the control signal is no longer applied (Fig 2). However, the predictability of evolution and adaptation of synthetic organisms is limited. Indeed, population-level dynamical adaptation, noise during gene expression and continuing reproduction need to be taken into account both at the design stage and as part of the experimental verification.

Biological systems are subject to high levels of both extrinsic noise and intrinsic stochasticity, owing to low copy numbers of DNA and mRNA [19]. Typically, feedback loops are used to attenuate the effects of noise. However, the level of noise in natural organisms far exceeds that of man-made systems. Furthermore, cells are able to use noise as a signalling mechanism. Contrary to typical engineering designs, biology exploits unavoidable variability to ensure robustness and adaptation to changing environments. Therefore, a synthetic design principle can either be to construct modules that are robust to noise or to explicitly use the stochastic nature of the cellular environment in the design process. In the former case, recent work [20] has addressed the fundamental limits of suppressing molecular fluctuation as a function of the signalling rate. Synthetic circuits might therefore require a hybrid solution that incorporates both design principles.

One of the most challenging aspects of synthetic biology is the engineering of evolution. The possibility that a designed biological component might in future generations cease to exist or, worse yet, might mutate into something different raises important ethical and practical questions. Evolution and mutation do not fit into any of the existing theoretical frameworks for robustness or modelling, although work in this direction has already begun both experimentally [21] and theoretically [22]. In the former case, randomized non-essential sequences were inserted into DNA sequences that code for a simple feed-forward loop. The resulting synthetic constructs still meet the design criteria, although the non-essential sequences were shown to have an effect on dynamic functionality. In the latter work, the authors developed a dynamic model that accounts for the adaptive behaviour seen in *E. coli* that fits with general evolutionary principles. From a conceptual point of view it might be helpful to think of evolution, mutation and adaptation as a type of discrete uncertainty, in a mathematical sense, that affects the long-term dynamics of a synthetic organism. In comparison, intrinsic and extrinsic noise could be viewed as short-term continuous effects.

In almost all natural cells, the genetic code contains redundant or at least non-coding sequences termed 'junk DNA'. In many cases, junk DNA contains transposons that cause mutations and genes whose function is unknown—possible relics from previous evolutionary steps. In keeping with the 'minimal genome' hypothesis, previous work developed an *E. coli* genome with much of this junk DNA removed [23]. It showed that the synthesized bacteria had exactly the same growth rate properties as the wild type. In theory, minimal genome organisms should also reduce the risk of mutation and evolution and provide a solid basis for enforcing modularity and minimal designs in synthetic biology.

It is vital that future design frameworks consider evolution, to ensure that synthetic organisms behave as predicted over longer timescales, and are thus compliant from a biosafety standpoint. In terms of moving synthetic biology from the lab to real world applications it is perhaps this challenge that most needs a solution.

From an industrial perspective, efficiency usually means yield maximization or minimal resource utilization. From a biological perspective, this utilitarian perspective—in an economical and mathematical sense—does not share the same objective as natural evolution. Natural biochemical pathways are optimized through evolution for their ability to adapt and not necessarily for a particular function, as is typically required for industrial applications. In addition, pathways often carry an evolutionary burden. Thus, design for industrial applications cannot borrow blindly from natural biochemical pathways. It is notable that robustness in the context of industrial applications does not have the

same meaning as robustness with respect to environmental release. For example, biofuel production [24] typically takes place in an industrial reactor, and the primary robustness issue is to maximize the fuel yield in the presence of varying food quality. In this example, the synthetic organisms do not interact with the environment in the same way as do synthetic organisms designed for other tasks, hence the importance of robustness to that uncertainty is not as high.

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Some of the most challenging problems for optimizing synthetic biology applications are those that try to blend network topology optimization with network flux optimization. Work towards this goal is described in [25], in which pathway optimization is carried out on a case-by-case basis. At the other end of the spectrum, the authors in [26] explicitly take into account optimization through pathway evolution, in a general mathematical and computational framework. Both approaches promise interesting and diverse solution strategies.

In spite of these fundamentally difficult engineering challenges, there have been several industrial-scale success stories. For example, the modification of natural biochemical pathways has been used for the production of heterologous proteins [25]. Similar applications for waste degradation [15] and biofuels [27] are under development. One of the biggest successes so far has come from Jay Keasling's lab at the University of California, Berkley, USA, which focuses on improving the production of the anti-malarial drug artemisinin [28]. The plant gene amorphadiene synthase (ADS) was introduced into a re-engineered native biochemical pathway of *S. cerevisiae* to achieve a high yield of artemisinin with low resource consumption. The promise to provide a cheap anti-malarial drug in developing nations constitutes a significant step in demonstrating the benefits of synthetic biology, to a potentially sceptical public.

From an environmental perspective, metabolic engineering has been successful in producing polylactic acid (PLA). PLA is a thermoplastic with many favourable properties: it is biodegradable, biocompatible and has low toxicity for humans. Furthermore, it is synthesized from renewable resources such as starch and sugar cane. Recent work [29] followed a full synthetic biology design cycle, including computational analysis and full genome-wide flux analysis, to develop a metabolic pathway that was inserted into *E. coli*. The authors knocked out unnecessary genes and were able to achieve an 11wt% increase in PLA synthesis from glucose, and up to 56wt% increase in its copolymers. As with artemisinin, these industrial examples offer much needed solutions to major societal challenges, and are a key ingredient in the necessary public engagement.

Slightly outside our definition of synthetic biology, another industrial scale success took place in 2010. The Cayman Islands Mosquito Research and Control Unit in collaboration with Oxitec announced the results of a large field trial, during which approximately 3 million genetically modified (GM) mosquitos were released with the objective of reducing the population of the mosquito *Aedes aegypti*, a vector of the dengue virus. In this instance the *A. aegypti* population dropped by 80% [30]. The approach involved the 'sterile insect technique' [31] and a method from molecular biology known as 'release of insects carrying a dominant lethal' (RIDL) to ensure the synthetic population died out over time [32].

The ability to create synthetic organisms, combined with our inability to control them with solid guarantees, raises the need to consider the ethical implications. Many of these issues are not new and have been addressed in many areas of life science research. We therefore particularly address the ethical considerations that relate to the introduction of synthetic organisms to the environment and describe a possible decision-making framework.

Among the most commonly raised ethical issues are those focusing on the potential for synthetic biologists to create life or concerns about 'playing God' [33]. Counterarguments specific to synthetic biology have been made by Douglas and Savulescu [34]. Another primary concern focuses on the fact that reductionist approaches to synthetic biology might erode the distinction between organisms and machines. Further issues concern the moral status of synthetic organisms in relation to natural living organisms and non-living matter [35].

However, in our view, the ethical issues that most warrant consideration relate to the possible risks of releasing synthetic entities into the environment, given that predicting their future behaviour is a challenging task. Whilst this should motivate synthetic biologists to improve design techniques, ethical analysis might help determine what level of predictability should be required, and how the possible risks should be weighed against probable benefits.

It is of paramount importance that designs should behave and evolve in a predictable manner. However, achieving perfect predictability and controllability is difficult. Thus, it is important to determine what risks are acceptable for releasing synthetic organisms into the environment.

One approach to deal with risk has been the precautionary principle, which has been used in areas as diverse as climate change, ozone depletion, nanotechnology and GM crops. There are differing formulations of the precautionary principle [36,37], but a theme common to many of these is that one should exercise caution, or even refrain from specific policies or actions, when the risks of a major catastrophe are present, even if very low. Such approaches are intended to draw attention to the deficiencies of standard decision theory when applied to events with very low probability.

The promise to provide a cheap anti-malarial drug in developing nations constitutes a significant step in demonstrating the benefits of synthetic biology, to a potentially sceptical public

A notable problem with the precautionary principle is that it seems to be inappropriately insensitive to the benefits of a course of action, or, put another way, to the risks of inaction [37,38]. Suppose that releasing a synthetic organism into the environment poses a significant but very low risk of serious ecological disruption, but it is almost certain to have marked benefits, such as curing a common and lethal disease, eliminating famine or substantially mitigating climate change. Strong variants of the precautionary

principle recommend against releasing this organism on the basis that there is a significant risk of a seriously bad outcome, but this seems an overly cautious approach. In this case, the benefits of releasing seem to outweigh the risks—indeed, the benefits include the elimination or mitigation of even more serious and certain risks.

In response to this and other problems faced by the precautionary principle, the concept of 'reasonable risk' has been proposed. In the context of releasing new organisms into the environment, the following factors are relevant for determining whether the risks are reasonable [39]. Is there a known direct risk to the welfare of sentient beings before releasing the new organism? What is its magnitude, based on evidence available at the time? Should any non-human, non-biological or epidemiological research, systematic overview or computer modelling have been performed before the study to better estimate the risk to sentient beings? Could the risk be reduced? Is it as small as possible? Are the potential benefits of this study worth the risks? Are there indirect risks to the welfare of sentient beings? For example, could the organism be captured, altered and used for destructive purposes by a malevolent agent?

…the ethical issues that most warrant consideration relate to the possible risks of releasing synthetic entities into the environment…

Each of these points should be addressed before releasing a synthetic organism into the environment. There are further questions about how these considerations should be weighed against each another. However, it is possible to make some schematic comments about this approach. For example, the level of robustness to unmodelled interactions and evolution, which must be engineered into a synthetic organism before release, depends on its intended location. Thus, minimum robustness needs to be considerably higher for organisms intended for release into the general environment than for organisms that are to be used in tightly controlled industrial settings. The level of minimum robustness required also depends on the probable benefits of the organism. A less stringent robustness guarantee would be required of organisms that would, for example, marginally increase the efficiency of highly specialized industrial processes that probably take place in a controlled industrial lab environment than of organisms that might substantially mitigate major global threats and must be released into the environment.

There are also further questions about who should be charged with applying the 'reasonable risk' framework: national-level professional bodies, individual research institutions and scientists, or companies? There might be good reasons for vesting the ultimate responsibility of decision-making in an international body. The constituent members of such a group would ideally include leading scientists in the field of synthetic biology in addition to an ethical, industrial and governmental component. First, this would help to avoid duplication of work and confer economies of scale. Second, given that the release of new organisms into the environment could have significant consequences for everyone, basic democratic principles would dictate that such a body should represent the interests of all. Third, releasing a new organism into the environment can have irreversible effects. If individual institutions separately assessed the reasonableness of risks and act on their own assessments, it would take only one institution to underestimate the risks in order for irreversible harm to be done.

As synthetic biology touches on many sensitive ethical questions, a dialogue between scientists, industry and the public is paramount to prevent misunderstandings about research. One example is the debate about GM food in the UK. It was primarily a communication issue that ended GM food production in the UK. As noted in [40], although members of the public are happy to take recombinant-DNA-based drugs such as insulin or interferon, foods with even trace amounts of recombinant DNA are viewed as highly offensive.

Researchers in synthetic biology need to explain the huge impact that the field might have on medicine and industry. Public concerns about safety and the ability of responsible self-regulation must be addressed [41]. If researchers are willing to show that measures such as decreased population fitness are being considered, and applied, then this can only help increase trust in synthetic biologists by the public. Ultimately, it is the dialogue between the research community and the public that can pave the way for acceptance of synthetic biology. This is only possible if communication between research communities, and the public, is established from the very beginning. In addition, industry can be valuable by drafting a code of practice and setting standards for biosafety and biosecurity.

Ultimately, it is the dialogue between the research community and the public that can pave the way for acceptance of synthetic biology

Synthetic biologists have already taken a proactive attitude by collaborating with social scientists and engaging with the public from the outset. For example, the four Research Councils in the UK (BBSRC, EPSRC, ESRC and AHRC) have created and jointly funded seven research networks that include researchers interested in the 'ethical, legal and social implications' (ELSI) of synthetic biology. These networks encourage regular meetings of synthetic biologists and ELSI researchers, informing both sides about technological and ELSI progress. Moreover, public engagement meetings that should ensure the correct interpretation of the biotechnological achievements, and future public acceptance, are strongly encouraged. The public should be informed, in particular, that synthetic biologists adequately take care of biosafety arrangements and, in so doing, allow the field to regulate itself [3]. To this end, the European Commission initiated the SYNBIOSAFE consortium (http://www.synbiosafe.eu/) focusing on the discussion of biosafety and ethical concerns, and facilitating a socially acceptable development in all related fields. Most importantly, however, scientists working in synthetic biology must effectively communicate and maintain an open dialogue with the public as the future of the field also depends on public approval. Synthetic biologists and ELSI experts should analyse these issues and offer well-informed governance recommendations.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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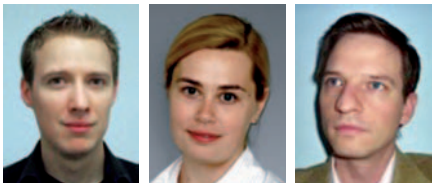

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