Safeguarding the Genetic Firewall with Xenobiology**

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Summary

While progress is being made in synthetic biology to make biology easier to engineer, the safety regulations and risk assessment practices will soon be rendered outdated and inadequate to handle upcoming developments of synthetic biology functions, organisms, and products. Xenobiology, the science of biological systems made out of alternative biochemical structures, may provide a new set of tools to establish an innovative solution, a genetic firewall, to future biosafety challenges. This genetic firewall will provide a stronger safety framework than would a series of small ad hoc fixes to a set of regulations designed for genetic engineering. Decisive and collaborative action by scientists, policy makers, and other stakeholders is needed to face the medium- to long-term biosafety risks of synthetic biology.

Current realities

The potential future release of deeply engineered or novel synthetic microorganisms raises the issue of their intentional or accidental interaction with the environment. Containment systems, risk assessment, and safety regulations designed for genetic engineering in the 1980s and '90s, for the purpose of limiting the spread of genetically engineered organisms and their recombinant traits, are still largely viewed by regulators and scientists as sufficient for contemporary synthetic biology products.

Progress in synthetic biology is expected to yield a staggering growth in the number of new biological functions and modified organisms with useful purposes. These developments will sooner or later pose a significant problem for established biosafety and risk assessment practices. A technological development that is outpacing its safety regulation is going to end up in (i) a series of unintended consequences and unforeseen accidents, (ii) a legal bottleneck for further product development because of a lack of a clear legal and regulatory framework, and/or (iii) increasing, and well justified, public resistance toward synthetic organisms if they are not considered to be "safe enough." So far, no safety mechanism is available to provide a sustainable solution to this dilemma. Past suggestions such as so-

called "suicide circuits" (that would kill the cell once it escapes into the environment) have failed to provide a sufficient degree of safety for industrial, medical, or environmental use. No strategy beyond the decades-old approach to biological containment is currently envisaged, despite significant investments and first successes in synthetic biology that have made biology easier to engineer.

Scientific opportunities and challenges

Most synthetic biologists try to convert biology into an engineering discipline by redesigning *existing*, natural biological systems for useful purposes. This means that synthetic biologists are using genes found in nature or designing new ones that closely resemble natural genes. Some bioengineers, however, are not satisfied with the biochemical substrates found in nature and try to construct new forms of life, called xenobiology, that have no counterpart in the extant world.

The synthesis of alternative biological structures focuses mostly on the three universal molecules: DNA, RNA and proteins. Recent research shows, for example, that all subunits of the DNA (base, deoxyribose, and phosphate group) can be replaced by alternative chemical structures. A DNA with three instead of only two base pairs has been made, and other carbon ring structures such as hexose or cyclohexenyl were incorporated to give rise to HNA and CeNA respectively (for all non-DNA, non-RNA molecules the term XNA is used, which stands for xeno nucleic acids). It was even possible to incorporate non-natural amino acids into proteins, so they are made up of 21 or 22 instead of 20 amino acid building blocks. First attempts have been made to come up with a biochemistry that has the opposite chirality of natural building blocks. So, instead of using left-handed proteins, future "mirror life forms" might use right-handed proteins. These experiments have mainly been carried out *in vitro* and very few if any (depending on the definition) living organisms exist with an altered biochemistry. However, sooner or later we will see the construction of new-to-nature or xenobiological systems that are increasingly farther away from their natural counterparts.

Xenobiology provides three main opportunities

1. Better understanding of the origin of life. Looking at all the possible alternatives to "life as we know it," the different variants of XNA, the hundreds of amino acids not used in proteins, the universal genetic code, or the selection of one type of chirality, the questions are: Why was this basic chemical make-up evolutionarily successful while others were not? Were these chemistries more robust, more likely to emerge under the

conditions of early Earth or was it by chance? And, is there room for an artificial biodiversity?

2. More efficient industrial biotechnology production systems. Although Earth has experienced billions of years of evolutionary trial and error, nature has (by far) not "tested" all possible biological systems. This means that it is likely possible to find new and more efficient biological functions than those provided by nature. This approach constitutes a promising way to design a new class of chemically diversified biocatalysts for industrial, medical, and environmental biotechnology. Industrial strains with a fundamentally different genetic code would suddenly be immune to natural phages or viruses.
3. A solution to the upcoming biosafety challenges. Xenobiological systems could be used as a fundamentally new biosafety system. Since they are not capable of horizontal gene transfer between the natural and new-to-nature organisms, the separation from the extant biology acts like a "genetic firewall" (see Figure 1).

While the first point addresses deep philosophical questions, the last two points deal with real-world implications for society, economy, industry, policy, and the environment. The use of xenobiology in industry, however, will primarily depend on whether it will be safe to use. Therefore, it ultimately comes down to xenobiology as a way to provide a genetic firewall to improve biosafety.

Constructing a xenobiological organism and a genetic firewall is a very difficult task, and beyond the current capabilities of science and engineering. Not only is it difficult, it might provide hardly any return of investment over the short-term future, since the establishment of a xenobiological toolkit and expertise will take some time before reaching a level that is remotely comparable to bioengineering with traditional biochemistries. But over the mid- and long-term the investment will pay off, both in terms of increased efficiency of biotechnological processes and in terms of providing a safe and reliable mechanism that avoids unintended consequences, accidents, and legal uncertainties.

Policy issues

Synthetic biology, extrapolated into the near future, will result in a radical increase in the number of synthetic biological functions, organisms, and products. Policy makers need to realize that these developments will render the current regulatory safety framework (designed for genetic engineering) outdated in a not-so-distant future.

- The response to this development may either be (i) inert, ad hoc, tactical adaptations of obsolete safety regulations and risk assessment without a clear strategy, or (hopefully) (ii) a collaborative approach that leads to a strategic vision of how to deal with upcoming safety challenges of synthetic biology, to avoid biological accidents, legal uncertainties, and safety-based public resistance. Adaptations may still be needed, but this time they would be based on a solid framework.
- The genetic firewall could at the same time improve industrial processes and establish safer biosystems, but only if a collaborative action among international scientists, policy makers, and other stakeholders can be established.
- Instead of resuscitating the limited concept of genetic suicide circuits, scientists, safety experts, and policy makers may discuss radical innovations as a strategic answer to upcoming biosafety challenges. The pros and cons of xenobiology and the support and deployment of the genetic firewall need to be discussed among international stakeholders.
- Decisive action to radically improve future biosafety issues is required from policy makers, concerned with the governance of biotechnology in Europe and the United States, in the form of (i) support to the technical development of a genetic firewall, and (ii) preparation of a regulatory framework that details in which circumstances the genetic firewall has to be deployed.

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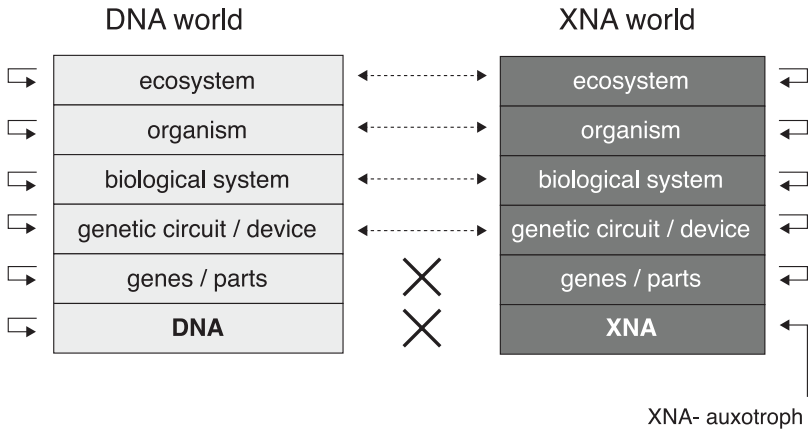
Figure 1: **DNA and XNA organisms would be able to interact on the level of whole organisms but would not exchange genetic material through horizontal gene transfer or via sexual reproduction (genetic firewall). Also, the XNA world needs to be completely dependent on external supply of essential chemicals that it cannot synthesize itself (XNA-auxotrophy).**



Debate summary

The following summary is based on notes recorded by the ISGP staff during the not-for-attribution debate of the policy position paper prepared by Dr. Markus Schmidt (see above). Dr. Schmidt initiated the debate with a 5-minute statement of his views and then actively engaged the conference participants, including other authors, throughout the remainder of the 90-minute period. This Debate Summary represents the ISGP's best effort to accurately capture the comments offered and questions posed by all participants, as well as those responses made by Dr. Schmidt. Given the not-for-attribution format of the debate, the views comprising this summary do not necessarily represent the views of Dr. Schmidt, as evidenced by his policy position paper. Rather, it is, and should be read as, an overview of the areas of agreement and disagreement that emerged from all those participating in the critical debate.

Debate conclusions

- Xenobiology, with its potential to reveal fundamental information about biology and the origin of life and even life on other planets, is a potentially transformative technology.

- The regulation of xenobiology, especially with respect to the safety of its procedures, its impact on human and environmental health and public safety, and its toxicity, presents significant challenges. Although much can be learned from the regulation of other biotechnologies, xenobiology will likely have unique aspects concerning the creation of a foolproof genetic firewall and the appropriate levels of physical and biological containment.
- Given the public concern over recent advances in genetic engineering, including the public, policy makers, and legislators in discussions characterizing the benefits and risks of xenobiology is critical to shaping effective policy.
- Concerns about public safety and security requires a level of governmental control of xenobiology that may not be welcome by those who wish to have open access to the technology and who promote its uninhibited, rapid innovation.

Current realities

Xenobiology was generally acknowledged as an early-stage scientific field, which is rapidly evolving. Although the assertion was made that xenobiology could create a "genetic firewall" that would protect the natural environment from synthetic organisms, this was strongly disputed. Given the extremely early stage of its development, there are numerous unknown aspects of xenobiology related to its safety, toxicity, and environmental interactions that were seen as potentially dangerous. Although XNA may not be able to interact genetically with natural DNA, XNA, or non-natural amino acids, may have a toxic effect on natural organisms by interacting with the DNA replication machinery, or through immune system effects. This would require additional layers of containment for any xenobiology experiments or production. Xenobiology was not regarded as a perfect firewall, and it was suggested that presenting it as such was an overstatement with potentially harmful consequences.

The premise behind xenobiology was also questioned: Given that organisms created via synthetic biology are already fragile, was there a need for xenobiology? If xenobiology could be shown to be safe and commercially viable, it could be a transformational technology.

Much of the debate centered on the appropriate way to regulate a new technology such as xenobiology under current regulatory frameworks. Historical parallels were drawn to the introduction of other major technologies, such as the

automobile or the airplane, where safety guidelines took some time to catch up with the demonstrated capabilities of the technology. To avoid similar lags in ensuring safety, it was proposed that regulations for xenobiology be implemented in advance. The key component in providing effective regulation is assessing risk, and while it is difficult to foresee all risks associated with such a new technology, several proposals were made (e.g., gaming, modeling exercises, risk registers) for methods to examine potential outcomes. Asking the right questions, and continuing to ask questions as the technology progresses, were seen as critical to accurately assessing risk. Carefully constructing a robust risk assessment framework was considered to be a more effective approach than regulation through legislation or government regulation alone. However, it was acknowledged that the complexity of xenobiological systems would make assessing their potential impact challenging, and require extensive testing and re-evaluation.

The public security questions involve determining who can be trusted to work on or have access to information about organisms derived from xenobiology, particularly if the fears about toxicity or autoimmune responses were realized. The potential validity of these concerns were acknowledged, but not seen as especially different from challenges being faced by the synthetic biology community, or for any other new, potential dual-use technology.

There was disagreement over whether conducting research into xenobiology would divert attention or resources away from research into proven, well-known natural DNA, RNA, or protein systems. Many technical issues remain to be resolved in these areas, including through synthetic biology, and proposing a shift toward xenobiology may distract from these efforts. However, industry is already doing xenobiology and will continue to do so. A particularly attractive aspect of xenobiology for industry would be the ability to "switch off" an engineered organism as a way to protect intellectual property (IP).

Scientific opportunities and challenges

Many of the challenges posed for xenobiology are the same as those being faced by synthetic biology. There was concern that the discussion was being framed as the two technologies in opposition to each other, whereas given the similarities between them, coordination would be more productive perspective. Creating division between the two may provide an opportunity for opponents of biotechnology to "divide and conquer" and limit progress in both fields.

A significant opportunity from xenobiology is the potential to provide information about fundamental questions of biology. If xenobiology shows that living organisms can be built with material other than the naturally occurring four

bases of DNA, the three base codon, or the naturally occurring 20 amino acids, the basic concepts of the origins of life would be challenged. Additionally, information gleaned from xenobiology research could provide details about how life evolved on Earth. Space agencies in the U.S. and Europe have taken a particular interest in xenobiology because of the potential to inform the search for life on other planets (exobiology). In the anticipation that life may one day be found elsewhere in the solar system (or universe), agencies such as the National Aeronautics and Space Administration (NASA) are using xenobiology as a model to determine how any extraterrestrial life should be handled (i.e., containment, worker protections, safety protocols) if it can be returned to Earth. There are also resultant issues about how to communicate with the public about any changes to our fundamental understanding of life, either as a result of xenobiology or exobiology.

Safety concerns around xenobiology were viewed as a significant challenge. Questions were raised concerning how organisms created via xenobiology would interact with other natural organisms in the environment, what effect (if any) these organisms would have on humans, whether these organisms or their products would bioaccumulate in the environment or be degraded, and how readily would they spread? Answers to these questions would be critical to making accurate risk assessments, but it will be challenging to test each parameter safely. Particular examples included the difficulty of assessing effects on humans with existing rodent models, and the published autoimmune reaction to some types of xenobiology products. Assessing these risks in theory or *in silico* is unlikely to be adequate.

The implication that xenobiology could provide a foolproof safety system for synthetic biology was also seen as problematic. It was stated repeatedly that nothing should be regarded as foolproof. Because it would be unwise to rely solely on xenobiology to "contain" a synthetic biological organism (i.e., prevent it from interacting with the environment), other physical containment systems would be required. In this respect, xenobiology likely would not alleviate many of the concerns around synthetic biology escaping from the laboratory or fermenter to interact with the environment, as it would be difficult to definitively test such interactions in advance of an escape. As a first step, preparing a detailed plan for evaluating and testing each aspect of the release (intentional or accidental) of a xenobiological organism would be important to provide some comfort to those worried about potential adverse effects on the environment or human health.

There were concerns expressed about the potential for industry to monopolize this technology as a way to simply protect its intellectual property. The example of terminator technology, or genetic use restriction technology (GURT), was raised. This technology would prevent farmers from using second-generation seeds from

genetically engineered plants, hence protecting the IP of the company that created the plant. A similar situation could occur with xenobiology, with the public and citizen scientists denied access to the technology should it be developed by big industry. This would raise a number of political, social, and ethical issues, and potentially lead to a backlash against xenobiology, similar to what occurred with agricultural biotechnology, especially in Europe.

A significant challenge in advancing xenobiology is the extent to which the public should be included in discussions about costs and benefits. The question remains as to how much effort should be devoted to acquainting the public with the terminology, potential, and pitfalls of the technology. The role of mass media (e.g., movies and documentaries) was raised as an illustration of how xenobiology could be presented to the public, either in a positive or negative light. The ability to use these media to either enhance public understanding and support, or seed fear in the public and lead to calls for limits or moratoria on research, were considered. It was felt that the public should be included in the discussion about xenobiology from the outset. Although there was the potential for creating a backlash, trying to exclude the public from the discussion would likely be more detrimental in the longer term. Achieving a positive outcome (i.e., having the public assess the technology in a rational way), would depend on the types of outreach and inclusion employed. It was generally acknowledged that public discourse about this subject would be difficult.

Policy issues

Preparing governance structures for xenobiology was presented as a challenging task, given the early-stage of the technology's development. It was acknowledged that excitement alone is not enough to warrant significant investment in the field. The field of xenobiology would need to mature before concrete progress could be made on regulatory systems. However, it was noted that xenobiology is already starting to show promise from a research perspective (e.g., using organisms to crack hydrocarbons, building enzymes from nonnatural amino acids, constructing a six base pair DNA to aid in HIV diagnosis). Some of these applications, although still at the demonstration stage, are particularly exciting because the reactions they catalyze cannot be performed in natural biological systems. Projects such as the European Union's Metacode program are already investing in such applications.

A substantial portion of the discussion involved the nuances of regulatory policy and how to apply different approaches to xenobiology. The regulatory guidance for synthetic biology as it stands in the U.S. is relatively unclear, and certainly not prescriptive in terms of conducting meaningful risk assessments.

Specifically, the guidance for synthetic biology research has been issued by the Office of Biotechnology Activities (OBA), which suggests that risk from a genetic element should be considered the same as the risk posed by the organism from which it came. This leaves a significant gap, as many synthetic biology genetic elements may be derived from no organism at all. Thus how can risk be meaningfully assessed?

This problem would likely be even more relevant in xenobiology, where the very components of such an element are completely new. There were some suggestions that approaches and analyses could come from the engineering field. While it was emphasized that asking the right questions was important, the issue of how to regulate such an unknown technology was largely left unanswered. Views differed as to whether the approach should be anticipatory (i.e., through regulation enacted in advance) or dynamic (i.e., by continually asking questions to shape the response).

Governance of xenobiology through self-regulation also was suggested. Some felt this term to be problematic, as it might suggest an unwillingness to have oversight from outside the community, so the alternative term "safeguards" was proposed. A project in 2007 examined attitudes of European scientists toward different governance approaches, and self-regulation was not a popular course of action, in contrast to the preference of U.S. scientists. This highlighted the need to provide culturally appropriate governance mechanisms.

Questions were raised as to who should control such genetic firewall technology. Specifically, it was proposed that such a powerful and potentially dangerous technology should remain under government control, although this would raise issues as to who should be given access and under what circumstances, and which governments should have control. Xenobiology, it was countered, needs to be an open source, publicly available resource, as this would allow for the greatest impact as a safeguard against misuse. Control and access are ultimately political issues that will need to be resolved at the political level.

Given the difficulty in communicating xenobiology to the public, there also would be issues concerning how to regulate xenobiology in communications with policy makers. It was cautioned that being seen to be asking for early regulation could result in overly restrictive controls, especially if the distinction was not made effectively among regulation, guidance, and frameworks. It was agreed that communicating this science effectively would be a key policy challenge as the field progresses.

Comments suggested that existing safety regulations were obsolete for dealing with a technology such as xenobiology. Regulatory systems vary widely among

different countries, and some nations, such as the U.S. and Australia, were viewed as having quite robust systems in place. Much could also be learned from the pharmaceutical sector, particularly in the area of self-regulation. Pharmaceutical companies have been dealing with the need to ensure safety for new products for a long time, and there may be lessons that can be extrapolated to xenobiology.

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Table of contents