Biochemistry and Cell Biology

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Journal:	<i>Biochemistry and Cell Biology</i>
Manuscript ID	bcb-2021-0340.R1
Manuscript Type:	Article
Date Submitted by the Author:	03-Sep-2021
Complete List of Authors:	Sheahan, Taylor; University of Lethbridge, Chemistry and Biochemistry Wieden, Hans-Joachim; University of Lethbridge, Chemistry and Biochemistry
Keyword:	Next-generation synthetic biology, biotechnology policy and regulation, cell-free systems, life-similar systems
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Emerging regulatory challenges of next-generation synthetic biology

Initiating a cell-free synbio policy discussion

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Abstract

Cell-free synthetic biology is a rapidly developing biotechnology with the potential to solve the world's biggest problems; however, this promise also has implications for global biosecurity and biosafety. Given the current situation with COVID-19 and its economic impact, capitalizing on the potential of cell-free synthetic biology from an economic, biosafety, and biosecurity perspective contributes to our preparedness for the next pandemic, and urges the development of appropriate policies and regulations, together with the necessary mitigation technologies. Proactive involvement from scientists is necessary to avoid misconceptions and assist in the policy making process

Keywords

Next-generation synthetic biology; biotechnology policy and regulation; cell-free systems; life-similar systems

Synthetic biology (synbio) has emerged as a scientific field with tremendous transformative potential, providing critical tools for achieving the sustainable development goals identified by the United Nations (UN) (United Nations 2020). Through the rational engineering of living systems, synbio-inspired biotechnology has the prospect to deliver alternative solutions to combat global food shortages, climate change, energy resource depletion, and therapeutic limitations (Kelly et al. 2014). However, current approaches depending on re-purposing and re-programming existing living systems (top-down) impose limitations that need to be overcome to harness the full potential of synbio. The bottom-up design of life-similar systems and devices that function outside the constraints of living cells (i.e. cell free synbio) side-step these limitations and are gaining traction in both academia and industry.

Life-similar systems provide new opportunities, but also present novel risks with respect to biosafety and biosecurity. Given its global impact, it is surprising that within current regulatory conversations the emerging risks and sharing benefits associated with cell-free and life-similar biotechnology are not discussed. Among policymakers and scientists there is a debate over what is defined as “novel biotechnology”, leading to confusion over the regulation of next-generation synbio and uncertainty regarding what policies and regulations apply to cell-free systems (Keiper and Atanassova 2020). This potential for omission is of concern due to the speed at which disruptive technologies are developed and adopted, and the scope of their impact. Here, we provide a forward-looking assessment of the biosafety and biosecurity implications of cell-free and life-similar systems, and promote a framework for global evidence-based regulatory conversations. Overall, we aim to support the continued translation of cell-free technology into products and applications, while also safeguarding public trust.

EMERGING ROLE OF CELL-FREE SYSTEMS FOR SYN BIO

The current, primarily top-down, approach to engineering biological systems has resulted in a variety of commercial successes ranging from artemisinin acid production in yeast for the treatment of malaria (Ro et al. 2006) to precise genome engineering tools such as CRISPR (Ran et al. 2012). However, the full potential of synbio has not yet been realized as integration with industrial infrastructure and medical practices has been challenging (Chen 2012; Chen and Smolke 2011), impacting its disruptive potential to provide novel approaches to produce biofuels, biomaterials, and therapeutics. This is in part due to the controversy surrounding genetically modified organisms (GMOs), their possible impact on the environment, and corresponding containment and regulation concerns. Further compounding is the lack of regulatory security regarding protection of intellectual property and limitations faced in the straight-forward designability of living systems counteracting their evident benefits (Figure 1).

As an alternative, cell-free systems provide life-similar functionality outside the constraints of living cells and are available, commercially or prepared in-house, as cell extracts or purified reconstituted systems. These systems, which include the necessary biomolecular machinery to produce RNA and protein, result in a reduced bio-contamination risk, as cell-free components are currently unable to replicate, propagate or evolve. Compared to cell-based approaches, cell-free systems are not limited by competing cellular processes, such as metabolism, and no cloning selection method is required. Since they are not restricted by experimental constraints, such as transformation efficiency, they enable rapid design-build-test cycles. Their ease of use, reduced complexity, ability to incorporate unnatural amino acids and tolerance of toxic compounds makes cell-free systems a powerful tool for synthetic biology and biotechnology development.

Consequently, cell-free systems have successfully been used to produce important biomolecules, including therapeutics (such as the cytotoxic protein onconase (Salehi et al. 2016) and medically applicable cannabinoids (Valliere et al. 2020)) and metabolites (including the industrially relevant monoterpenes (Korman et al. 2017)), demonstrating their commercial relevance as bio-factories. Outside their use as a platform technology, these systems enable the development of bio-devices with applications in diagnostics (such as the paper-based detection of Zika virus (Pardee et al. 2016)), bioremediation, and biosensing. Overall, cell-free systems provide a promising avenue for developing solutions to the grand challenges synbio aims to address. Their potential is especially evident when considering the negative public perception of GMOs. For example, cell-free approaches to bioremediation circumvent the need to release a genetically modified organism into the environment, providing a “safer” alternative, as well as aligning with well-established customer and production models.

With continued advancement, the application of cell-free systems is becoming mainstream and is not limited to a classical laboratory setting. Ease of use and inherent safety allow cell-free synbio to be more accessible to the public and citizen scientists in comparison to conventional biological engineering of living systems, especially with growing trends in do-it-yourself (DIY) biology and the establishment of community labs. For example, BioBits™, an educational kit developed using cell-free systems brings synbio to the classroom, lowering the barrier for schools to use these technologies and moving towards more mainstream uses of synbio (Stark et al. 2018).

In recent years, cell-free based start-up companies have emerged, such as Arbor Biosciences (Arbor Biosciences 2021) and Sutro Biopharma (Sutro Biopharma 2021), which use cell-free approaches for protein engineering. Recently, SwiftScale Biologics have pivoted use of their cell-free platform technology to combat the COVID-19 pandemic by rapidly producing

antibodies (Swiftscale Biologics 2021). Utility of cell-free systems in times of crisis was also demonstrated by the Broad Institute during a pressure test conducted by the U.S Defense Advanced Research Projects Agency (DARPA). When infrastructure and current production methods are compromised and cell-based approaches are not feasible due to toxicity issues, cell-free systems can be engineered to rapidly produce important biomolecules (Casini et al. 2018). Overall, this demonstrates use for on-demand fabrication of necessary biomolecules and medicines.

Most recently, bottom-up approaches to synbio have received considerable attention, providing an alternative route to addressing the challenges faced by engineering living systems (and derived cell-free approaches) to unlock the full potential of synbio (Schwille 2011). By taking a reverse engineering approach, biological processes are refactored and reconstructed from scratch (i.e. refactoring life). Specific cellular functions are separated from the complexity of living organisms, allowing for the rational design of life-similar synthetic biological systems. Advancements in bottom-up synbio towards the ultimate goal of creating synthetic life (see the Build-a-Cell initiative (Build-A-Cell 2021)) have consequently opened the door to new biotechnology created outside of the confines of living cells and the engineering limitations imposed by the design strategies applied by living systems (e.g. genetic code and codon usage, biophysical properties, etc.). Life-similar systems build on experience made with conventional cell-free systems; they are not affected by the inherent complexities of cellular processes that have evolved to enable fitness of the cell under a wide variety of stresses and conditions, providing engineers with increased control and greater freedom of design. This is especially true in the case of purified reconstituted systems, which consist of a reduced number of specified components required to execute select life-similar functions outside of a cell.

BIOSECURITY AND BIOSAFETY – THE POTENTIAL FOR MISUSE

As cell-free technology evolves and becomes more commonly accessible, it is important to acknowledge the dual-use potential for these systems and their latent ability to cause harm, intentionally (i.e. biosecurity risks) or accidentally (i.e. biosafety threats), as is the case with any other human technology. Along with this comes the need to implement proper policy and practices, and to develop new approaches to prevent and mitigate their misuse.

The emerging biosecurity risks (e.g. bioterror) associated with synbio technology developments was highlighted in a report by the National Academies of Science, Engineering, and Medicine for the U.S. Department of Defense (National Academies of Science, Engineering, and Medicine 2018). The properties of bottom-up cell-free systems that make them a promising platform technology for advanced bioengineering also bear dual-use potential that can be exploited by malicious parties, including non-state actors. The current economic impact of COVID-19 demonstrates that such an act of bioterrorism does not have to cause a large number of fatalities to significantly impact society. Streamlined open-source approaches and protocols to purify cell-free systems (e.g. one-pot purification methods (Lavickova and Maerkl 2019)) also simplify how they can be obtained and distributed, demonstrating the decreasing technology barrier, which is a hallmark of disruptive innovation potential. Furthermore, the genetic information required to program cell-free systems is easily accessible due to the low cost of DNA synthesis. With continued commercialization and increasing access, it is reasonable to predict at-home use of cell-free systems in the future. How these systems will be engineered (i.e. with increased stability), deployed, and interact with the environment may have important implications for biodiversity requiring a full assessment of the emerging risks.

The ability to incorporate unnatural amino acids and/or re-design the genetic code creates the potential to construct new-to-nature and undetectable biomolecular devices. Previous work has already achieved expansion of the genetic code from a four to eight letter alphabet, and progress has been made towards reassigning codons to encode for non-standard amino acids (Hoshika et al. 2019; Lajoie et al. 2013). Executing these novel codes in a cell-free system will evade current regulatory and screening regimes used to identify potential threats, impacting current global biosecurity measures.

Adding another layer of complexity, incorporation of artificial self-replication and self-regeneration into these reduced systems to create fully synthetic bio-machines or assemblies addresses a current shortcoming of cell-free systems. In 2019, the construction of a photosynthetic artificial cell capable of regenerating energy that was used to produce its own membrane proteins was reported, providing a step closer to creating a completely independent artificial system (Berhanu, Ueda and Kuruma 2019). Progress in this area will be driven by commercial interests as these systems can utilize a proprietary genetic code (such as the eight-letter alphabet) to protect intellectual property of the design and/or the unauthorized manipulation of these systems. Concerns of creating a deadly bioengineered “organism” that will impact humans on a population scale is now amplified by the ability to create orthogonal synthetic systems that could impact all forms of life and escape classical detection and mitigation strategies.

INNOVATION VS. REGULATION

Uncertainty with respect to regulation of cell-free synbio exists, especially regarding orthogonal synthetic life-similar systems, impacting both the application and full utilization of the technology, and the social licenses to do so. Reflecting on the current public opinion of GMOs reveals a

disconnect between what scientists consider to be safe and what the public views as dangerous. Proactive involvement from scientists is necessary to avoid such misconceptions and assist in the policy making process. Developing the appropriate regulations, including guidelines, practices, and standards, is important because it will affect how the technology is used globally and facilitate the necessary risk assessment by governments and insurance companies to ensure biotechnology investment, which is a critical component for capitalizing on the promise of cell-free synbio. This raises the question: *where do cell-free synbio and life-similar systems currently fit in the regulatory landscape?* More importantly, *how does one govern emerging technologies, while ensuring safety and security without preventing global access to the resulting benefits?*

Cell-free systems and the technologies developed to build them might be considered enabling technologies covered under the *seven experiments of concern*, which were identified in 2004 by the National Academies of Science, Engineering, and Medicine. These experiments, including those that facilitate evasion of being detected, were recommended to undergo “review and discussion by informed members of the scientific and medical community before they are undertaken or published”, due to their impact on biosecurity (National Research Council 2004). Seventeen years later, the application of cell-free synbio further increases the plausibility and probability of these potential threats; however, it has not been addressed so far.

Currently, there is a critical opportunity to develop the appropriate guidelines to reduce the risk associated with their use. If we consider industrial revolutions of the past, it becomes evident that each revolution is more innovative than the previous and occurs much more rapidly (Sorensen and Brocchini 2021). In the “age of hyper-innovation”, where disruptive and transformative technologies are developed and adopted quickly, there is less time for the public to adjust. Thus, it is imperative that we make informed decisions during these initial stages of technology

development to shape how, for example, cell-free synbio is incorporated into our society. This includes a global strategy to address and manage innovation through regulation, best practices, and training, including conversations with relevant industry sectors, government agencies, and academic institutions. Recognizing both the potential benefits and threats early in the course of development will enable sustainable growth while providing safety and security without stifling innovation.

Core Security and Safety Actions

In considering the safety and dual-use potential of cell-free systems, it is important to consider ways to identify and counter possible threats and safety concerns, an approach that likely is true for any emerging and disruptive technology. **First**, this should include a technology *co-development initiative* that aims to create corresponding biotechnology to mitigate against misuse. For example, when creating a novel genetic code, methods and technology tools to experimentally de-code encrypted sequences can be developed in parallel. Bioinformatic tools capable of decoding encrypted sequences can help to identify potential threats that may not be recognized using current screening methods, which aim to prevent the synthesis of known viral or toxic genes (using the list of Human and Animal Pathogens and Toxins for Export Control developed and maintained by the Australia Group (The Australia Group 2021)). Additionally, applying a safety-by-design approach, such as the design of fail-safe or on/off switches for orthogonal synthetic life-similar systems integrated into the technology development pipeline, would be beneficial to safeguard against their intentional or accidental release. Such technology co-development if mandated by funders, publishers, institutions and governments will help to encourage scientists throughout the

design-build-test process to assess and prepare for alternative ways in which their research may be applied and demonstrate to the public proactive risk management.

Secondly, it is important to develop a global *technology co-assessment* culture and to provide the required tools to all involved parties. Common practices of technology assessment will be critical for an evidence-based, open and inclusive global conversation about the use of cell-free and life-similar technologies. Given the exponential development speed, it is important to enable and facilitate the co-creation of sustainable regulation at the point of technology inception. The implementation of this hinges on the ability of the participating groups to assess the potential for unintentional uses of cell-free and life-similar systems, as well as a foundational ethical framework. Current debate over what is considered “novel biotechnology” has led to some exclusion regarding risk assessment and policy development, further highlighting the need to have these critical conversations. With this in mind, structures need to be established that include technology assessment training and awareness alongside related scientific training, for example in high schools during the initial stages of synbio education (i.e. the International Genetically Engineered Machine competition) (Dubé et al. 2017). Society as a whole will greatly be affected, both positively and negatively, by the potential benefits and risks, thus it is necessary for the effective adoption of norms and guidelines around cell-free synbio, that these guidelines are inclusive, and that they can be implemented by emerging economies with less developed regulatory or monitoring systems and institutions.

It is proposed that proactive security and safety measures, including mitigation strategies and surveillance tools, be discussed, co-developed and implemented immediately on a global scale to protect against the misuse of cell-free systems, in contrast to developing regulations once it is too late, especially considering the risk associated with a potential population wide event. Given

the current situation with COVID-19, the economic impact in response to the pandemic potential of a manmade non-lethal virus urges the development of appropriate policies and regulations, together with the necessary mitigation technologies. These measures are important to realize the full potential of synbio and corresponding emerging technologies, and to not encounter a situation where advancement of cell-free and life-similar technology is delayed or stopped due to overregulation and lack of social licence. Getting this right will not only improve implementation and acceptance of the guiding principles but will also enable leapfrogging into the affected technology space and facilitate the corresponding global sharing of benefits.

STARTING A GLOBAL REGULATORY CONVERSATION

At both national and international levels, there are a variety of policies in place that aim to reduce biosecurity risks and minimize biosafety threats. Specific agencies focus on different aspects of developed biotechnology, while the degree of regulation varies extensively between countries (National Academies of Sciences, Engineering, and Medicine 2016). This results in a complicated and disjointed regulatory framework with the potential for gaps or loopholes. On the part of researchers, this also leads to an administrative burden as they struggle to identify and comply with relevant regulations.

Currently, the Convention on Biological Diversity (CBD) focuses on the impact of synthetic biology including gene editing and gene drive technology at an international scale (Convention on Biological Diversity 2000), while the U.S. Department of Defense is concerned with bioterrorism in the form of engineered superbugs (National Academies of Science, Engineering and Medicine 2018), and other agencies discuss the general impact of GMOs. Within these policies, it is unclear where cell-free systems or emerging life-similar technologies fit, and

there is no discussion of their potential benefits and risks. While most protocols govern the use of living modified organisms, this term does not cover cell-free and synthetic life-similar systems (Convention on Biological Diversity 2019). With non-living systems receiving considerably less attention and only mentioned briefly, it is a missed opportunity to proactively assess the risks and benefits of cell-free and life-similar technology.

To amend this lack of inclusion and overcome current issues of a disjointed regulatory framework, it is suggested that an international organization, such as the World Health Organization, UN, or the CBD, assesses the potential biosecurity risks associated with orthogonal cell-free synbio and life-similar systems. Given that it is not always easy for existing programs within these organizations to adjust their focus in response to the rapidly changing biotechnology landscape, there is an opportunity to create an independent body to evaluate the biosafety of next-generation synbio. Such a program could be supported by a not-for-profit organization and operate under the umbrella of one of these larger institutions, where it has the potential to complement existing programs by interfacing with related activities (e.g. the UN Biological Weapons Convention, the CBD Cartagena Protocol on Biosafety). This will require involvement from scientists to provide the necessary technical expertise to inform policy development.

The need for global approaches to assess the impact of emerging biotechnologies, such as cell-free and life-similar systems, is recognized by the Nuclear Threat Initiative, Biosecurity Division (NTI:Bio), which has convened the Global Biosecurity Dialogue in Addis Ababa in 2019 (supported by the African Union and Global Affairs Canada). NTI:Bio identified emerging biological risks caused by developing technology as one of their major work streams, resulting in the Biosecurity Innovation and Risk Reduction Initiative (BIRRI). Last year they released a report with the World Economic Forum recommending improved DNA screening procedures to “prevent

illicit DNA synthesis and misuse” (World Economic Forum 2020). More recently, NTI:Bio and the BIRRI continued discussions virtually building towards the establishment of a global entity focused solely on biosecurity risk reduction. A main priority within this initiative is the creation of standards that will assist funders, grantees, and publishers in the identification of biological risk, improving risk mitigation (NTI 2021). In line with these efforts, it would be beneficial for such an organization to conduct a review of cell-free technologies, assisting in addressing the regulatory challenges that lie ahead. For example, how does one classify a self-replicating artificial cell and what impact does compartmentalization have on regulation? Should cell-free systems be regulated differently depending on their use or application? How does one assess the technology that enables advances with respect to dual-use? How does orthogonality of these systems impact detection and removal of harmful bio-agents produced? Does the enabling technology or end-product belong to its country of origin and how does this impact who has access to the benefits provided by the technology?

As cell-free synbio matures, it is necessary to develop the appropriate safety and security measures, while recognizing the exciting new developments made possible by this area of bioengineering. It is our responsibility to encourage global societal discussions on important scientific and technical advances and seek social licencing and consent early in the process. Due to the immense potential of cell-free biotechnology and the likely role it will play in addressing global problems in the future, inclusion in the regulatory conversation will ensure responsible use and preparedness while also fostering innovation.

Acknowledgements

We thank Fabian Rhoden and Emily Wilton for helpful discussion and comments on the manuscript.

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Figure 1: Progression of synthetic biology advancement. At each stage of synthetic biology, the corresponding level of applications, supporting technology, accessibility, safety and security risks, and regulation is provided as a heat map. Synthetic Biology 1.0 refers to the early stages of bioengineering utilizing a primarily top-down approach, including cellular and genetic engineering; Synthetic Biology 2.0 refers to advancements that utilize both top-down and bottom-up synbio approaches, such as strain engineering, minimal cells, and artificial cells; Refactoring Life refers to the next phase of synbio, including the development of cell-free and life-similar systems through a primarily bottom-up approach. This phase is hallmarked by an increased potential of orthogonality, as well as the potential to escape from traditional monitoring and regulations applied to living systems.

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