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A Legal, Philosophical and Biological Analysis

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Biological Entity

A Legal, Philosophical and Biological Analysis

TU Delft

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A Legal, Philosophical and Biological Analysis

Commissioning Organization
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Summary

Recent advancements in synthetic biology fundamentally challenge the traditional definition of an ‘organism’ and the regulatory use of the term ‘biological entity’. Developments such as gene editing, organoids, DNA computing, and synthetic cells increasingly blur the boundaries between living and non-living systems, prompting critical reassessment of foundational biological and legal concepts.

Current European and Dutch legislation, including Directive 2009/41/EC and the GMO Decree, defines organisms as "*biological entities capable of replication or of transferring genetic material*". However, the term ‘biological entity’ itself remains undefined. Scientific advisory bodies like COGEM (the Netherlands Commission on Genetic Modification) have long recognized that synthetic biology renders current definitions inadequate. Their reports consistently highlight how novel biological constructs, such as synthetic cells and biohybrid systems, do not align with traditional concepts of life, yet demand regulatory oversight.

Additionally, while the terms ‘organism’ and ‘biological entity’ are both used in law, their meanings can diverge significantly in scientific discourse. Legal definitions are often structured around risk and containment rather than biological accuracy, creating friction between regulatory objectives and scientific realities. For example, while scientists often exclude viruses from the category of organisms due to their host-dependence, legal frameworks classify them as biological entities, and thus subsequently as organisms for risk management reasons.

In this report, we investigate the concept of biological entity to provide greater clarity and conceptual structure to debates concerning what constitutes, or what ought to constitute, an organism. Through contextual analysis in three domains – legal, philosophical and biological – we arrive at a definition for a biological entity and criteria for assessment. To arrive at such, we develop two sets of criteria, i.e., Line of Thought (LoT) – LoT 2 being complementary to LoT 1, but not vice versa – to help classify ambiguous biological innovations on whether these should be labeled as a biological entity:

LoT 1: A composition-based perspective derived from current biological and philosophical literature, using criteria such as biological composition, functional integration, self-organization, and a stable internal structure;

LoT 2: An interaction-based, forward-looking perspective, developed with expert input, emphasizing the entity’s potential to act, including its environmental context, potential to interact with living systems, and the extent of controllability or reversibility.

Subsequently, these LoTs are both applied to three illustrative borderline cases in synthetic biology, provided by the Ministry of I&W: (i) infectious RNA,(ii) Lipid Nanoparticles with self-replicating RNA, and (iii) minicells. Also, we explore whether, and how, we can distinguish biological entities between naturally occurring and synthetic entities.

Finally, based on contextual analyses, interviews, a focus group and application of defined criteria to the provided cases, we arrive at the following definition of a biological entity: “*any material construct, system or component, regardless of origin or current activity, that has the actual or potential capacity*

to interact meaningfully with living systems or facilitate biological processes in an ecologically or physiologically relevant context".

Hereby, the following connotations are made:

- "any material construct, system or component" includes 'traditional' organisms, viruses, genetic constructs, synthetic (proto)cells, RNA particles, engineered proteins and more.
- "regardless of origin or current activity" avoids making a distinction between natural and synthetic, and accounts for some entities being inert under laboratory conditions but can become active under specific conditions.
- "to interact meaningfully with living systems or facilitate biological processes" shifts the focus from structural traits like metabolism or replication, to functional relevance.
- "in an ecologically or physiologically relevant context" ensures that interaction potential is not considered in the abstract, but in relation to real-world conditions.

Such definition and criteria could allow regulators to better anticipate emergent behaviors and novel risks, particularly from synthetic constructs designed to be more modular, passive, or 'non-living' in traditional terms.

Contents

1 Introduction

Developments in the field of synthetic biology present challenges to the consistency of the concept of 'organism' as used in regulation, specifically the notion of 'biological entity' which functions as the primary criterion for determining what counts as an organism. Techniques such as gene- and cell therapy, gene editing, and DNA computing, along with the development of organoids, cultivated meat, and specialized drug delivery systems, represent only a fraction of the field's transformative potential. Advancements in synthetic biology have led to the creation of entities that challenge traditional definitions of 'life', prompting a reevaluation of what constitutes a biological entity.

To ensure the safe and responsible use of genetically modified organisms (GMOs) within its member states, the European Union (EU) has established a comprehensive framework of directives that regulate their development, release and commercialization. GMO Directives 2001/18/EC (deliberate release into the environment) and 2009/41/EC (contained use) both define the term '(micro-) organism' using the concept of a 'biological entity'. The Directives provide the following definitions: (i) micro-organism: any cellular or non-cellular *microbiological entity* with the ability to replicate or to transfer genetic material, including viruses, viroids and animal and plant cells in culture; (ii) organism: micro-organism or other *biological entity* with the ability to replicate or to transfer genetic material. In the Netherlands, the Dutch Ministry of Infrastructure and Water Management (I&W) has adopted the EU definition of (micro)organism in its national GMO Decree[1]. However, regulations concerning GMOs and synthetic biology hinge on how an 'organism' is currently defined, in which the term 'biological entity' centers prominently. Notably, this term does not yet have a universally accepted definition. However, vast developments in the fields of biotechnology and synthetic biology call for clarification on used definitions to monitor and regulate respective fields responsibly.

Several advisory bodies in Europe monitor and signal on synthetic biology developments. In the Netherlands, the Commission on Genetic Modification (COGEM) has, since 2006, pointed out that synthetic biology may give rise to organisms that fall outside the scope of current GMO risk assessments. Its reports highlight blurred boundaries between life and non-life, as well as the emergence of applications without natural reference organisms, potentially rendering existing biosafety definitions obsolete (COGEM, 2006 – 2024). Similarly, the German Zentrale Kommission für die Biologische Sicherheit (ZKBS) has monitored developments in synthetic biology such as minimal cells, xenobiology, and protocells in the light of biosafety. While most applications still fit within current laws on genetic engineering, the ZKBS does note that future synthetic, self-replicating systems without natural comparators may no longer be covered by existing legislation, requiring new risk assessment criteria[2]. Both advisory bodies emphasize that synthetic biology is pushing the limits of current definitions, particularly of what counts as an organism, and stress the need for regulatory adaptation.

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[1] Article 1.5 of 'Besluit genetisch gemodificeerde organismen milieubeheer 2013', via https://wetten.overheid.nl/BWBR0035090/2019-07-01

[2] ZKBS, June 2018. 2nd interim report of Synthetic Biology, accessible via https://zkbs-online.de/fileadmin/user_upload/Downloads/Fokusthemen/Synthetische_Biologie/2nd_report_Synthetic_Biology_2018.pdf

Reports from COGEM and the ZKBS illustrate the need to update and redefine *biological entity* and associated notions to remain fit-for-purpose as the boundaries of biotechnology are constantly being pushed. Traditionally, the distinction between living organisms and non-living matter was based on characteristics such as self-sustaining metabolism, reproduction, and the ability to evolve. Living organisms exhibit homeostasis, respond to stimuli, and undergo cellular processes essential for growth and survival, whereas non-living matter lacks these dynamic properties, for instance, a stone. However, synthetic biology creates entities that blur these boundaries by integrating biological and synthetic components, such as engineered cells, protocells, and biohybrid systems, challenging conventional classifications. As the field continues to expand its influence across multiple disciplines and ecosystems, there is an increasing need for regulation and communication regarding its societal, health, and environmental implications (Little, 2024). Regulatory updates are necessary to ensure a promising future for synthetic biology within the European Union and the Netherlands – one that supports innovation, ensures the safety and health of all organisms, and promotes environmental sustainability.

One of the key challenges in achieving regulatory clarity lies in the absence of a shared, consistent, and operational definition of what constitutes a *biological entity*. Without such a definition, it becomes difficult to determine whether certain synthetic organisms fall within existing legal and biosafety frameworks. This difficulty is rooted in long-standing debates within the philosophy of biology, where the meaning of *biological entity* is often inferred from the broader context of the literature, rather than explicitly defined. As a result, the term remains conceptually ambiguous. Additionally, the rise of synthetic biology adds a new dimension to the debate, making the boundaries of the term even more blurred.

This report aims to arrive at a shared definition and criteria for a ‘biological entity’ through analysis from three contexts associated with the term:

1. **Legal Context**: How are ‘biological entity’ and closely related notions mentioned in the EC Directives 2001/18 and 2009/41, as well as in national regulation and outside of Europe? What interpretation can be drawn from its use in these legal texts? Additionally, what insights can be derived from other related regulations where this term is applied?
2. **Philosophical Context**: From a philosophical standpoint, is there a way to conceptualize the relationship between ‘organism’ and ‘biological entity’? Can these terms be reconciled or distinguished through philosophical analysis?
3. **Biological Context**: How can the concept of a ‘biological entity’ be defined based on current biological knowledge? In what ways does the term ‘biological entity’ correspond to the defining characteristics of an organism, particularly in the light of developments in synthetic biology?

Reader’s Guide

To structure our conceptual inquiry and guide the reader through our reasoning, this report develops two distinct Lines of Thought (LoTs). These LoTs represent successive analytical steps in our effort to explore and refine the definition of a *biological entity*. Taken together, the two LoTs provide a structured reflection of our analytical process and serve as the foundation for the definition of a biological entity proposed in this study. To support the reader in following the development of these two LoTs, our report is structured as follows.

After this introduction, **Chapter 2** details the methodological approach and outlines the conceptual research framework that underpins the study. This study is conducted through an extensive literature review focusing on three contextual domains, as stated in the section above, interviews and a focus group. In **Chapter 3**, findings from the three contextual analyses are presented. These findings form the empirical and conceptual foundation upon which our first Line of Thought (LoT 1) is developed, on which we elaborate in **Chapter 4**. This chapter also critically explores the applicability of LoT 1 to a hypothetical distinction between *naturally occurring* and *synthetic entities*. However, this analysis reveals that LoT 1 may not sufficiently resolve the definitional issues of a biological entity and may even introduce additional complexity for regulation and monitoring. Building upon, **Chapter 5** focuses on the development of a second Line of Thought (LoT 2), shaped by insights obtained through a focus group session. LoT 2 takes into account the limitations identified in LoT 1 and aims to offer a more refined set of criteria for defining a biological entity. In **Chapter 6**, we provide an extensive discussion in which both LoT 1 and LoT 2 are evaluated using the case studies provided by the Ministry of I&W. This comparative analysis helps assess the applicability and robustness of the proposed criteria in the LoTs, in real-world examples. **Chapter 7** concludes the study by proposing a definition of a biological entity based on the case studies (Chapter 6), outlining corresponding criteria, offering recommendations for future research, and their application in regulatory and safety contexts. In this chapter, we also reflect on the implications of the study and outline potential avenues for future research.

2 Method

Research presented in this report was conducted through literature review, a BSc thesis on how the term ‘biological entity’ is currently used in the biological and philosophical context, interviews and a focus group. The sections below provide more detailed information on the literature study for each respective context, i.e., legal, philosophical and biological, and additional data collection.

Legal Context

Analysis of the term ‘biological entity’ in its legal context started with analysis of the respective European GMO directives and the GMO decree, by means of the EUR-Lex website and the Dutch government website on biotechnology, i.e.,’Besluit genetisch gemodificeerde organismen Milieubeheer’ and ‘Regeling genetisch gemodificeerde organismen’. The website of the Dutch government mentioning EU legislation and the GMO Decree, also mentions other associated law and regulations, such as the Cartagena Protocol, GMO Labeling and GMO exceptions for COVID-19 research. These were also analyzed consecutively and examined on their applicability and relevance for this particular study. Additionally, to supplement the initial findings with other legislation and regulation that also mention ‘entity’, ‘biological entity’ or ‘natural entity’, ChatGPT (OpenAI, GPT-4, June 2025 version) was used to generate an overview of potentially relevant Directives, regulation and law within, and outside of Europe. These suggestions were subsequently manually reviewed, cross-checked and validated through Google, Google Scholar, Web of Science, and/or countries’ respective government websites. For a final check, Google Scholar and Web of Science were used for a search on the terms ‘natural entity’ to see whether more countries, additional to Canada and Australia, have discussions devoted to adopting this notion into law and regulation. The analysis provided in Section 3.1 is the author's original work.

Philosophical/ Biological Context

To define the term ‘biological entity’ a scoping literature review was conducted to explore key concepts, types of evidence, and research gaps. Definitions related to ‘biological entity’, such as ‘organism’, ‘replication’, and ‘genetic material’, were provided by the Ministry of I&W. The research was carried out using Google Scholar, Web of Science, and NIH databases. Searches were conducted between November 11, 2024, and March 6, 2025, using the following search terms:

- "Biological entity"
- "Biological entity" AND characteristics
- "Biological entity" AND "Biological individuality"
- "Biological entity" AND "Biological identity"
- "Biological entity" AND Organism OR Organicism
- "Biological entity" AND "Philosophical biology"
- "Biological entity" AND Ontology
- "Biological entity" AND "Biological objects"
- "Biological entity" AND "Biological systems"
- "Biological entity" AND "Synthetic Biology"

Articles were selected based on their titles and abstracts. The snowballing technique was also employed, in which the references of relevant articles were reviewed for additional sources. Inclusion criteria included studies and books published between 1999 and 2025, written in English, and available through the TU Delft and Leiden University databases. Exclusion criteria comprised duplicate articles, letters to the editor, and articles focusing on biological entity recognition techniques or machine learning applications. This resulted in 19 relevant academic books or papers. Titles and abstracts from the search results were analyzed, and relevant articles were further examined for their results and discussions. The quality of the sources was assessed based on the authors' credentials, high-quality references, the publication source, and the frequency of citations by other researchers.

Interviews

As part of the qualitative research methodology, semi-structured interviews were conducted to collect primary data. For the philosophical and biological analysis, three interviews were conducted in both English and Dutch between January 6 and January 17, 2025. These interviews focused on the relationship between 'organism' and 'biological entity', the defining characteristics and boundaries of biological entities, and the connection between biological entities and synthetic biology. The interviews began with general questions about the topic, followed by follow-up questions based on the responses given by the experts. To ensure unbiased responses, the proposed definition of 'biological entity' was introduced at the end of each interview, once the interviewees' views had been fully explored. A fourth interview was conducted in Dutch on June 27th, and focused on the legal definition and respective context of a biological entity as stated in Dutch and European Law and regulation.

The interviewees were selected for their expertise, varying from technical knowledge to law. The list below provides a brief profile of the interviewees:

- Interviewee 1 [INT1] is a researcher in cell biology and synthetic systems, an active member of the national EVOLF program (Evolving Life from Non-Life), and teaches courses in Nanobiology and Applied Physics.
- Interviewee 2 [INT2] is an assistant professor in Biotechnology and teaches in the Life Science and Technology program. The research draws inspiration from nature's designs for sustainable applications, particularly for industrial microbiology.
- Interviewee 3 [INT3] is a PhD candidate who has a background in physics and is conducting research on yeast cell division under the supervision of interviewee 1. This person's work extensively uses microscopy to observe cellular processes, providing a more quantitative perspective on the topic.
- Interviewee 4 [INT4] is an associate professor specializing in law and biotechnology.

Focus Group

Findings from the legal, biological and philosophical analysis were also discussed with experts in the field by means of a focus group. A dual goal was set for the focus group: (1) validate preliminary results from the conducted literature review on the definition and associated notions from a legal, philosophical and biological perspective, and (2) gain complementary insights into establishing criteria for what could define a biological entity.

The focus group was held physically, in English, on Thursday, June 19, 2025, with a total of 5 participants at Delft University of Technology. All participants are PhD candidates from TU Delft, with the exception of one master's student. All are studying/working with synthetic biology applications, or

associated domains, and the master's student being enrolled in 2 programs, i.e., Bionanoscience and Philosophy.

Case Studies
Three case studies were provided by the Ministry of I&W that illustrate the current ambiguity in assessing whether 'something' can be deemed a biological entity. These cases entail (i) Infectious RNA, (ii) LNP Particles with selfreplicating RNA, and (iii) minicells for dsRNA crop protection. Chapter 6 on the content of each respective case study and are all three analyzed in line with two 'sets' (lines of thought, LoT) of established criteria for defining a biological entity – also see 'methodological approach' below.

Ethics Statement
This study adheres to the ethical research standards of TU Delft's Human Research Ethics Committee (HREC). Prior to data collection, interviewees and participants of the focus group received via email an informed consent form that stated the research purpose, emphasized voluntary participation, ensured anonymity in data processing and withdrawal rights. Participants were encouraged to ask questions at the start of the interviews and focus group, and only proceeded after consent was given. Three out of four interviews were conducted physically. These were recorded and transcribed verbatim. The fourth interview was conducted online and transcribed automatically via Microsoft (MS) Teams. Recordings and transcripts were stored in the research team's secured TU Delft MS Teams folder, which is only accessible by the researchers of this study. In compliance with GDPR and university protocols, all data will be permanently deleted upon completion of this report.

Research timeline and methodological approach
Figure 1 (below) presents a chronological overview of the research process, outlining the key steps taken and the corresponding findings at each stage. The study began with an extensive literature review, establishing the philosophical and biological context necessary for framing the concept of a biological entity. Building on this foundation, three expert interviews were conducted, which led to the formulation of four preliminary criteria (LoT 1), the drafting of a midterm report and a first analysis on the provided case studies. Additionally, it was explored whether distinguishing between 'naturally' occurring, and synthetic entities would enable future-proof regulation in the light of technical developments (Chapter 4).

Subsequently, the focus shifted to examining the legal context. Additionally, a focus group session was hosted in which we critically evaluated the initial four criteria (i.e. LoT 1). This interactive discussion helped refine the developed criteria. As a result of these reflections, complementary criteria (LoT 2) were developed (Chapter 5). Lastly, outcomes from the legal context and the criteria developed in LoT 2 were assessed through an interview with an expert specializing in law and biotechnology.

Ultimately, all steps taken in this research led to the identification of two distinct sets of criteria (i.e. LoTs), offering deeper insight into how biological entities might be understood in the light of synthetic biology applications. These are:

1. Assessing an entity on its functional autonomy, biological agency, evolutionary potential, and structural and functional integration.
2. Focusing on what an entity does or can do in specific environments.

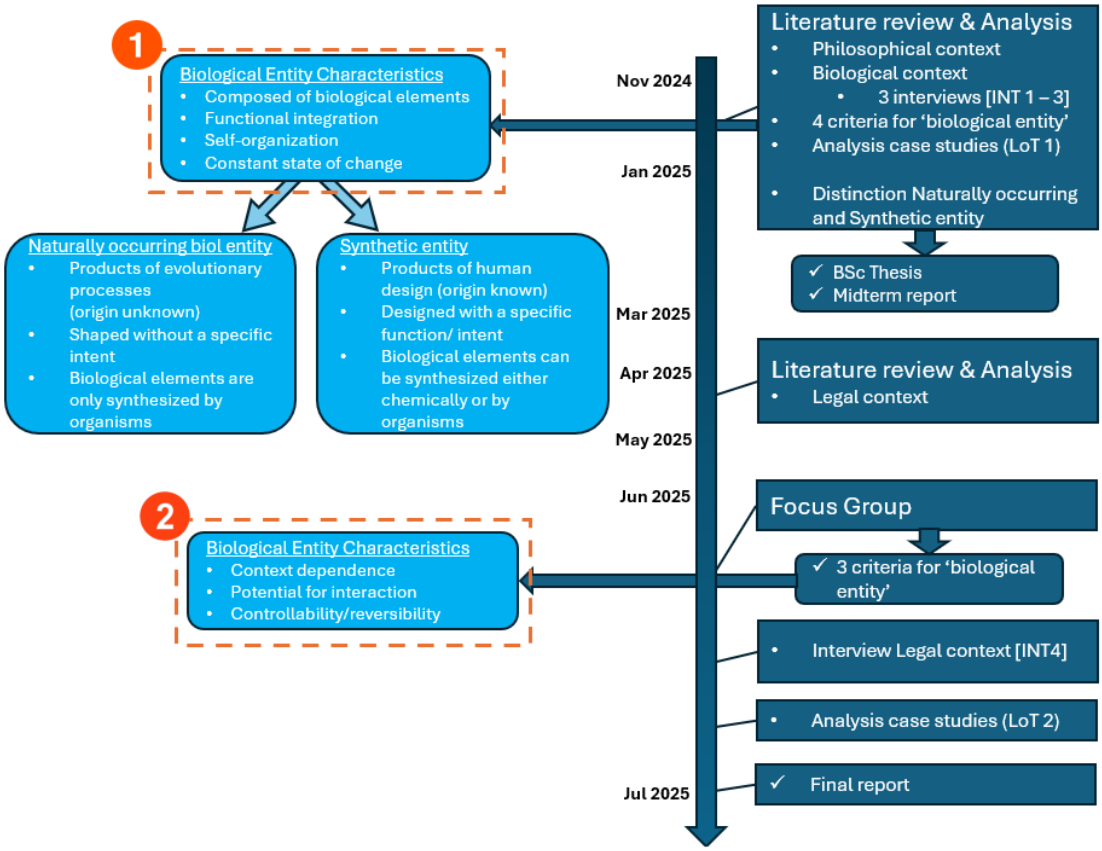

Figure 1: Overview of research timeline and key findings. This timeline illustrates the steps of this research process, beginning with a literature review on the philosophical and biological context and additional interviews, leading to 4 basic criteria for a biological entity (line of thought, LoT 1, box 1 in orange). Following upon, these criteria were analyzed in the context of synthetic biology applications, and the distinction was made between naturally occurring, and synthetic entities. Thereafter, the legal context was analyzed, followed by a focus group in which the criteria of LoT 1 were discussed, leading to three alternative criteria for determining a biological entity (LoT 2 – box 2 in orange). As a last step, criteria derived from the focus group and results from the legal context were discussed in a fourth interview.

3 Contextual Analyses

To define the term 'biological entity', several key aspects are examined. This includes its usage in legal contexts, its relationship to the concept of an organism in scientific and philosophical literature, and the fundamental characteristics that define a biological entity. In the following sections, we first introduce and define biological entity in its current legal context; within Europe, nationally, and outside of Europe. Thereafter, an analysis is provided how the term is applied within the fields of philosophy and synthetic biology, highlighting different aspects of this term. The perspectives of interviewed experts are integrated in the literature review, adding critical insights.

3.1 Legal Context

In biotechnology regulation, defining the legal interpretation of biological concepts is essential to ensure that laws are interpreted consistently with scientific realities and are capable of effectively safeguarding human health and the environment. In biosafety laws, for instance the EC Directives 2001/18[3] and 2009/41[4], *biological entity* serves as a determining factor for what constitutes an *organism,* namely "a biological entity with the ability to replicate or to transfer genetic material". The sections below provide an overview of 'biological entity' across different jurisdictions, focusing on the European Union (EU) and the Netherlands, and providing insights from other countries such as Australia, the United States, and Canada.

Biological Entity in European Law

While the term 'biological entity' is not explicitly defined in EU legislation, there are several regulations and Directives that implicitly refer to this term. GMO Directives 2001/18/EC (deliberate release into the environment) and 2009/41/EC (contained use) both refer to an entity by means of 'organism'. In detail, the EU directive for deliberate release in the environment states that an organism is "any biological entity capable of replication or of transferring genetic material". The directive on contained use relies on a more extensive description, specifically "including viruses, viroids, and animal and plant cells in culture". The EU deliberately included biological entity in its legal definition of an organism because it is species neutral[5], technology-neutral (Sandin, Munthe & Edvardsson Björnberg, 2022) and avoids having to list every possible life-form. This implies that, rather than using structural classifications, the EU adopted a function-based approach that ensures that a wide range of 'living' organisms can be regulated properly (Sprink et al. 2016). Thereby, it was assumed that this framing would be resilient to scientific innovation and would allow for a broad coverage of current and future biotechnologies.

However, the implicit definition of a 'biological entity' – centered on 'living' systems capable of replication or gene transfer – does not fully capture the range of constructs emerging in synthetic biology. For instance, synthetic gene circuits used in cell-free systems can carry out biological functions without being part of a living organism and without the ability to replicate. As such, they may fall outside the scope of current GMO regulations. This raises important questions about how to

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[3] https://eur-lex.europa.eu/eli/dir/2001/18/oj/eng
[4] https://eur-lex.europa.eu/eli/dir/2009/41/oj/eng
[5] Directive 2001/18/EC explicitly states "'organism' means *any* biological entity capable of replication or of transferring genetic material". This phrasing is thus *species-neutral* because it applies to *any* biological entity.

assess and govern such constructs, especially since they may still pose biosafety risks through unintended interactions with natural systems. Additionally, in some cases, concerns may arise around biosecurity, e.g., potential misuse or dual-use research, illustrating the increasing disconnect between legal definitions and technological developments.

The European Court of Justice (ECJ) has addressed such issue in cases as C-442/09[6] (Bablok and Others v Freistaat Bayern) through ruling that any living material capable of passing on genetic traits, *regardless of being a full organism or a derivative product* like pollen, would fall under the GMO Directive. This established that the *functional capability* of the material, rather than its method of production or 'completeness' as an organism, determines its legal applicability. By this, the ECJ underscored that environmental and genetic risks, not taxonomy or synthetic origin, are the decisive factors in determining what should be considered a GMO. This risk-based approach also reinforces the precautionary principle embedded in EU law, especially for environmental release scenarios or food safety risks. However, as this is a specific interpretation of how we should deal with engineered biological entities and does not necessarily follow from the current definition of a biological entity, this creates room for legal uncertainty.

Other, affiliated regulations and directives touch upon the term biological entity, but do not use the term specifically. In the text of the Nagoya Protocol on access to genetic resources and the fair and equitable sharing of benefits arising from their utilization (2010)[7] – a supplementary agreement to the Convention on Biological Diversity (CBD) – the term itself does not appear. However, the conceptual scope of the Protocol does overlap with the kinds of living organisms typically referred to as biological entities in biosafety or biotechnology law. For instance, in Article 2 of the CBD[8], '*genetic resources*' refers to "genetic material of actual or potential value", and '*genetic material*' referring to "any material of plant, animal, microbial or other origin containing functional units of *heredity*". These references implicitly cover the notion of 'replication or transferring of genetic material'. In addition, European countries that implement the Nagoya protocol (Regulation 511/2014[9]) often rely on existing definitions used in GMO Directives, i.e., the "utilization of genetic resources". So, although the Protocol serves a different purpose than, for instance, the GMO decree, the term biological entity is still used as a so-called regulatory proxy – a distinct but related, legal framework or regulation that guides or informs the application of a different set of rules or regulations.

Similar to the Nagoya Protocol and the CBD, many EU Regulations, Directives and Rules refer to the term explicitly or implicitly, e.g., as a regulatory proxy. Table 1 below provides an overview of such legal instruments within the EU, including the GMO Directives that anchor the term biological entity. Thereby, it should be noted that the EC Biotechnology Patent Directive 98/44[10], Rule 26(3) refers to "biological *material*", and not '*entity'* and states "… or being reproduced in a biological system" in addition to "being capable of reproducing itself…". The word 'entity' being left out of the Directive's main legal text is done intentionally as patent rules only care about tangible material that can be reproduced in a lab, and not about whether it is still 'alive'.

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[6]https://curia.europa.eu/juris/document/document.jsf;jsessionid=92F808CA40E71D8202A87BC3E1AF892E?text=&docid=109143&pageIndex=0&doclang=EN&mode=lst&dir=&occ=first&part=1&cid=6915040
[7] https://www.cbd.int/abs/default.shtml
[8] https://www.cbd.int/convention/articles?a=cbd-02&utm_
[9] https://eur-lex.europa.eu/eli/reg/2014/511/oj/eng
[10] https://eur-lex.europa.eu/eli/dir/1998/44/oj/eng

Table 1: Overview of EU Directives, Council Decisions and Regulations that use the term 'biological entity' and the term's contextual meaning.

Subject	***Leading EU Act***	***Specific phrasing 'biological entity'***	***Context***
Deliberate release of GMOs (introduction into the environment)	Directive 2001/18/EC (replacing Council Directive 90/220/EEC)	Article 2(1): "'organism' means any *biological entity* capable of replication or of transferring genetic material"	Anchors the whole risk-assessment system: only living, self-replicating entities trigger the Directive.
Contained use of GMOs	Directive 2009/41/EC	Article 2(a): "'micro-organism' means any *microbiological entity,* cellular or non-cellular, capable of replication…"	Same idea as deliberate release but fitted to lab conditions (contained use). The prefix *micro-* was added to signal that only microscopic biological entities are covered.
EU ratification of the Cartagena Biosafety Protocol	Council Decision 2002/628/EC[11]	Annex A, Article 3h: "'Living organism' means any *biological entity* capable of transferring or replicating genetic material, including sterile organisms, viruses and viroids".	Mirrors the universal Protocol text so that EU and international definitions stay identical.
Plant protection and biocides	Regulation 1107/2009/EC[12] on plant-protection products, and Regulation (EU) 528/2012[13] on biocides	1107/2009/EC, Chapter 1, Article 3(15); 528/2012, Chapter 1, Article 3(1b). Both Regulations define "micro-organism" as "any *microbiological entity*,…, capable of replication or of transferring genetic material".	Implements the biosafety definition into chemicals law so that products containing living organisms, such as *Bacillus thuringiensis*[14], are regulated like chemicals.
Zoonoses monitoring	Directive 2003/99/EC[15]	Article 2(b): "'zoonotic agent' means any virus, bacterium, fungus, parasite or other *biological entity* which is likely to cause a zoonosis".	Enables the zoonosis list to be open-ended; if a new pathogen is a *living* biological entity, the monitoring kicks in.

The legal use of biological entity has been instrumental in enabling countries to regulate genetically modified and synthetic organisms under a precautionary, function-based framework. This approach, grounded in biological function rather than taxonomy, supports regulatory consistency across jurisdictions.

[11] https://eur-lex.europa.eu/eli/dec/2002/628/oj/eng
[12] https://eur-lex.europa.eu/eli/reg/2009/1107/oj/eng
[13] https://eur-lex.europa.eu/eli/reg/2012/528/oj/eng
[14] *Bacillus thuringiensis* (or BT) is a bacterium used to kill certain insects such as tobacco budworm, beetles and some flies and mosquitoes. The BT-toxin crystal protein is present as inactive protoxins in the bacteria, making it usable as an insecticide. Some GM crops, like BT maize or corn have been genetically altered to produce this specific toxin, making them resistant to pests and herbicides.
[15] https://eur-lex.europa.eu/eli/dir/2003/99/oj/eng

Dutch Interpretation and Usage of Biological Entity

In the Netherlands, the GMO Decree primarily implements several key EU directives and regulations. The term biological entity is translated and implemented through a combination of national legislation and ministerial regulations. Dutch legal texts refer to biological entity as 'biologische entiteit', and retain the broad, function-based definition found in EU law. The Dutch implementation of Directive 2001/18/EC uses the EU's phrasing in regulatory documents related to environmental release permits for GMOs and does so too for Directive 2009/41/EC on contained use. In these regulations, the following definition is used: "micro-organism: any cellular or non-cellular micro-biological entity capable of replication or of transferring genetic material, including viruses, viroids and animal and plant cells in culture" (Article 1.5 of Besluit genetisch gemodificeerde organismen milieubeheer 2013[16]). Additionally, biological entity is also referred to in the national Working Conditions Decree, Article 4.84 (2a)[17] (in Dutch: Arbeidsomstandighedenbesluit) through the term of 'biological agents' (in Dutch: biologische agentia). These are defined as "micro-organisms, including those which have been genetically modified, cell cultures, and human endoparasites, which may be able to provoke any infection, allergy or toxicity". In line with EU Directive 2000/54/EC[18], the definition is integral to occupational health and safety regulations, in particular concerning the classification and handling of biological agents in the workplace, for instance, a laboratory.

Moreover, the Netherlands has emphasized alignment with EU risk assessment practices through its implementation of Regulation (EU) No 528/2012[19] (Biocidal Products Regulation) and Regulation (EC) No 1107/2009[20] (Plant Protection Products Regulation). In these domains, a biological entity includes any viable microorganism used as an active ingredient, highlighting the term's operational importance in Dutch biotechnology and environmental safety law. The Dutch Board for the Authorisation of Plant Protection Products and Biocides (in Dutch: College voor de toelating van gewasbeschermingsmiddelen en biociden, Ctgb) incorporates this term into its technical evaluation frameworks. These evaluations consider whether a living micro-organism (e.g., bacteria, fungi, viruses) intended for agricultural or biocidal use fits the criteria for regulatory scrutiny under EU and national laws. This ensures that the term biological entity continues to anchor microbial safety assessments within Dutch product authorization procedures.

Lastly, the term 'biological entity' is proposed to be referred to in the Dutch Embryo Act, i.e. in the third evaluation of the Act (2021)[21] and the initiative bill 36 416 (Explanatory memorandum, 2023)[22]. Herein, it is proposed to broaden the scope of an embryo's definition, referring to an embryo as a "biological entity resulting from the fusion of a human egg cell and a human sperm cell, in all stages of embryonic development". In this context, a biological entity's definition is central to the regulation of human embryonic research and reproductive technologies in the Netherlands. Recent evaluations suggest revising the definition to encompass entities formed through alternative methods, such as embryo-like structures (Cave, 2025), reflecting advancements in reproductive science.

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[16] Translated from Dutch. Original phrasing: "micro-organisme: elke cellulaire of niet-cellulaire microbiologische entiteit met het vermogen tot replicatie of tot overbrenging van genetisch materiaal, met inbegrip van virussen, viroïden en dierlijke en plantencellen in cultuur". See Article 1.5 of Besluit genetisch gemodificeerde organismen milieubeheer 2013, accessible via https://wetten.overheid.nl/BWBR0035090/2019-07-01

[17] https://wetten.overheid.nl/BWBR0008498/2025-02-01#Hoofdstuk4

[18] Article 2a in Dutch: "„biologische agentia": micro-organismen, met inbegrip van die welke genetisch zijn gemodificeerd, celculturen en menselijke endoparasieten die een infectie, allergie of toxiciteit kunnen veroorzaken" via https://eur-lex.europa.eu/eli/dir/2000/54/oj/eng

[19] https://eur-lex.europa.eu/eli/reg/2012/528/oj/eng

[20] https://eur-lex.europa.eu/eli/reg/2009/1107/oj/eng

[21] https://www.rijksoverheid.nl/documenten/rapporten/2021/03/15/derde-evaluatie-embryowet?utm_

[22] https://www.tweedekamer.nl/downloads/document?id=2023D37322&utm_

Biological Entity Beyond Europe

Australia directly uses the term biological entity in its legislation, particularly within the Gene Technology Act 2000[23], which defines an organism as "any biological entity that is viable, or capable of reproduction, or capable of transferring genetic material". This definition is consistently applied across all states and territories, establishing a harmonized national biosafety framework overseen by the Office of the Gene Technology Regulator. As in Europe, Australia has also adopted a function-based regulatory model – focusing on what the entity *can* do, e.g. reproduce, rather than what it *is*, e.g., a species, cell type – that ensures that a wide variety of GMOs and synthetic biology constructs fall within the scope of Australian biosafety law. Beyond biosafety, the terminology has also been extended into Australian bioethics legislation. The Prohibition of Human Cloning for Reproduction Act 2002[24] refers to a human embryo as "a biological entity with a human nuclear genome or altered human nuclear genome", and to a human embryo clone as “a genetic copy of another living or dead human, but does not include a human embryo created by the fertilization of a human egg by human sperm”. This phrasing is designed to ensure that synthetic or cloned embryos, even those not derived from fertilization, are covered by law. It illustrates the legal system’s proactive stance in adapting to emerging technologies in reproductive science.

In the United States, the term biological entity played a central role in federal biosafety regulation, particularly under Act 7 CFR § 340[25], which until its 2020[26] revision defined an organism as "any biological entity capable of replication or of transferring genetic material." This definition guided decades of regulatory oversight by the United States Department of Agriculture and informed GMO evaluations under the Coordinated Framework for the Regulation of Biotechnology. In Canada, the term biological entity does not appear explicitly in statutory language, but it is embedded in the regulatory logic of several key instruments, for instance under the Canadian Environmental Protection Act[27] and the New Substances Notification Regulations[28]. These frameworks mirror the language of the Cartagena Protocol on Biosafety, using functional criteria to define living organisms as those capable of replication or of transferring genetic material. This operational definition informs the work of agencies such as Environment and Climate Change Canada and the Canadian Food Inspection Agency. These bodies assess GM micro-organisms and ‘plants with novel traits’ (PNTs) to determine whether they fall under federal regulatory oversight. Although the phrase biological entity itself is not used, its meaning underpins biosafety risk assessments and regulatory categorization. Canada’s approach, therefore, maintains conceptual alignment with international standards while allowing flexible implementation. As in the EU and Australia, this framing supports effective oversight of both conventional GMOs and emerging synthetic organisms, though legal uncertainties persist around cell-free technologies and non-replicative constructs.

In Summary

The term *biological entity* serves as a foundational legal concept in biotechnology regulation. Although there is no definition found of biological entity itself, it is part of the definition of an organism and is generally used in connection with the characteristics of such: *capable of replication or transferring genetic material*. This function-based approach, used across the EU, Australia, Canada,

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[23] https://www.legislation.gov.au/C2004A00762/2016-07-01/text
[24] https://www.legislation.gov.au/C2004A01081/latest/text
[25] Archived on https://www.ecfr.gov/current/title-7/subtitle-B/chapter-III/part-340
[26] https://www.wiley.law/alert-USDAs-New-Rule-Modernizing-the-Regulation-of-Biotechnology-A-Practical-Legal-Summary
[27] https://laws-lois.justice.gc.ca/eng/acts/c-15.31/
[28] https://www.canada.ca/en/environment-climate-change/services/canadian-environmental-protection-act-registry/general-information/fact-sheets/new-substances-notification-regulations-organisms-2020.html

and historically in the U.S., ensures flexible oversight of genetically modified and synthetic organisms. The terms *organism* and *biological entity* appear to be closely related in EU legislation, which leads to important regulatory challenges. In Directive 2001/18/EC, an *organism* is defined as “any biological entity capable of replication or of transferring genetic material”. This definition is central to determining what falls under GMO regulation. New biological constructs such as cell-free systems, synthetic gene circuits, or protocells may carry out biological functions or express genetic material without meeting the criterion of being alive or capable of replication. As a result, they may fall outside the legal definition of an organism and therefore escape regulatory oversight – even when they pose potential biosafety or biosecurity risks. This highlights a growing mismatch between legal categories and technological realities.

In addition, the ruling of the ECJ has emphasized that the decisive criterion for classifying something as a GMO – and thus as an organism, and by extension a biological entity – is the risk it poses to the environment and to genetic integrity, rather than its taxonomic classification or method of production. This focus on environmental and genetic risk reinforces the EU’s precautionary principle and supports a functional, rather than origin-based, approach to regulation. It also signals a legal shift away from ontological definitions and toward risk-centered evaluation, particularly relevant for emerging applications involving synthetic or non-traditional biological systems. Thus, although *organism* and *biological entity* may appear similar, their legal distinction and the ambiguity surrounding the latter creates uncertainty in the regulation of emerging biotechnologies. Integrating risk as a guiding principle offers a more adaptive and consistent legal framework, ensuring that biosafety governance remains responsive in the face of accelerating innovation.

As a final point, to further contextualize the challenges surrounding the definition of biological entity, we also conducted a supporting analysis of the related concept *natural entity* – a more recent term that has been gaining political momentum for recognizing ecosystems, rivers and landscapes as rights-holders. Although this analysis lies beyond the main scope of this study, it can offer valuable conceptual insights. It also highlights that legal frameworks do not need to be grounded solely in scientific facts, but can also reflect interpretive judgments about nature, intentionality, and human intervention, revealing how legal systems adapt to evolving scientific developments and environmental values. Given its potential relevance for the development of new governance frameworks, a more detailed analysis is provided in Appendix A.

3.2 Philosophical and Biological Context

Besides the legal contextualization, it is crucial to also understand what the relationship is between a biological entity and organisms from a philosophical perspective. In this part we will explicate the relation between organisms and biological entities and identify where these concepts overlap and where they differ. In general, biological entities as a category encompass both organisms and other biological units. Findings in this section have led to the establishment of LoT 1 (see Chapter 4), assessing an entity on its functional autonomy, biological agency, evolutionary potential, and structural and functional integration.

Organisms

Organisms are at the core of biological classifications. An etymological analysis of this term reveals that ‘organic’, referring to natural organization, emerged in the late 17th and early 18th centuries. The suffix –‘ism’ denotes a distinctive practice or system, indicating that ‘organism’ in its literal sense

refers to a structured form of organization adapted for natural contexts (McConwell, 2023). The term 'organism' is used to establish hierarchical distinctions across different levels of biological organization (Pepper & Herron, 2008). Different conceptualizations of 'organism' exist in both scientific and philosophical contexts. The reductionist view sees an organism as the sum of its components (Wilson, 1999), whereas the processual view considers organisms as dynamic processes evolving over time (Morgan, 2021). Although the definitions of an organism remain ambiguous in both philosophical and scientific literature, there is a set of characteristics that are often considered to determine what an 'organism' is (Directive 2009/41/EC). These include autonomy, reproductive ability, cellular organization, metabolism, homeostasis, adaptability, and response to stimuli. (Fowler et al, 2023). These characteristics are inherent to the quality of being 'alive' typically associated with organisms. Biological entities, in contrast, can also exist in forms that are not considered 'alive'. For instance, proteins are widely recognized as biological entities in scientific literature (Vidal Arenas, 2024), but they are not considered 'living' as they cannot reproduce.

The category of *biological entities* includes both living organisms and non-living biological components. These entities are classified across multiple ontological levels of hierarchical organization. Broad or overlapping terms such as cells, organelles, organisms, species, and ecosystems are commonly used to describe biological entities at different levels (Vidal Arenas, 2024). At each level, biological entities engage in processes, possess properties, and consist of physical matter (Vidal Arenas, 2024). In this sense, biological entity functions as an *umbrella term* that spans molecular structures, cellular and organismal components, entire organisms, and ecosystems involved in biological processes (see Figure 2).

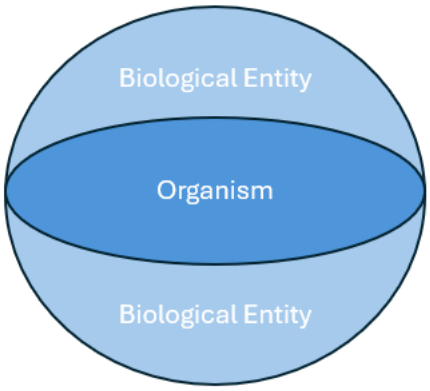

Figure 2: Graphical representation of the relationship between a biological entity and an organism.

Organisms, Biological Agency, and Autonomy

Autonomy is a key characteristic that distinguishes organisms from biological entities. In addition to the ability to replicate or transfer genetic material, organisms are also characterized by their functional autonomy. Functional autonomy refers to the ability to carry out essential life processes independently, such as metabolism, regulation, and adaptation to environmental changes (van Regenmortel, 2010). This autonomy distinguishes organisms from other biological entities that may rely on host systems or external environments for their functionality. Additionally, organisms exhibit biological agency, meaning they can perceive environmental cues and respond through directed actions, a feature that strengthens their classification as distinct biological entities (Okasha, 2023).

Components of organisms – such as organelles or specialized cell types – play integral roles in biological function but are not independently viable. These sub-organismal entities contribute to an organism's ability to maintain homeostasis and interact with its environment, yet they do not individually satisfy the criteria of functional autonomy. For example, mitochondria, despite containing their own genetic material, are dependent on their host cell for replication and essential processes (Okasha, 2023).

Evolution and Its Applicability to Biological Entities

Another defining characteristic of organisms is the capacity to undergo evolution via natural selection (McConwell, 2023). Evolution is broadly defined as the gradual change in the heritable traits of organisms over successive generations (Akkerhuis, 2024). While organisms and populations undergo evolution in this way, biological entities at lower organizational levels, such as organelles and biomolecules, experience modifications through mutation, degradation, or molecular evolution, but not through independent reproduction. According to [INT1], changes at the sub-organismal level, such as structural modifications in proteins or the accumulation of mutations in DNA, do not constitute evolution in the same way population-level genetic changes do. Similarly, [INT2] states that for an entity to truly evolve, it must be capable of self-replication; otherwise, any changes that occur in its structure or function cease when the entity itself is no longer viable.

Despite this distinction, smaller biological entities can contribute to evolutionary processes. The theory of symbiogenesis provides an expanded perspective on evolution beyond traditional natural selection, where independent biological entities merge to form more complex life forms. A key example of this is the evolutionary origin of mitochondria and chloroplasts, which originated from free-living prokaryotic organisms that established a mutualistic relationship with early eukaryotic cells (Gontier, 2016). Over time, these endosymbiotic relationships led to the integration of these entities as essential organelles, reshaping the complexity of life.

4 LoT 1: A composition-based perspective on Biological Entity

Building on the preceding contextual analyses, the first Line of Thought (LoT 1) offers four characteristics of biological entity grounded in established biological and philosophical literature. As illustrated in Figure 2 (Section 3.2), biological entities, and so also the derived characteristics, encompasses molecular structures, cellular components, individual organisms, and even entire ecosystems. Despite differences in scale and complexity, these entities share key organizational and functional properties (Vidal Arenas, 2024).

This approach emphasizes intrinsic characteristics commonly used to describe and distinguish biological systems, including biological composition, functional integration, self-organization, and the presence of a stable internal structure (see Table 2). These criteria reflect a structurally oriented perspective that highlights internal coherence and autonomy – an understanding aligned with traditional scientific and philosophical views. Based on literature review and expert interviews, LoT 1 identifies a set of core features that can help define the scope of biological entities.

Table 2: Composition-based perspective on characteristics to define a 'biological entity' based on current usage, definitions, and context.

LoT 1: Biological Entity Characteristics			
Composed of biological elements	Functional integration	Self-organization	Constant state of change

4.1 Composition and Biological Origin

A fundamental characteristic of biological entities is their *composition from biological elements*. Biological elements are chemical compounds structured through biological processes into complex macromolecules. As [INT1] explains, "A biological entity must, to some extent, be produced by a biological organism. This excludes purely chemical substances such as water and salts but includes biomolecules like lipids, as they are synthesized through biological processes". [INT2] concurs, emphasizing that a biological entity includes anything synthesized by a living organism, either the organism itself or its biologically derived components. This definition excludes inorganic byproducts such as ethanol, which may result from biological activity but do not inherently possess biological functions.

Although molecules such as water and inorganic compounds contain the same atomic elements as biological macromolecules, they are not structured through biological processes, nor do they independently participate in biological functions. While essential for life, they act as passive contributors rather than forming active biological entities.

Functional Integration and Self-Organization
Two interrelated characteristics that further distinguish biological entities from non-biological entities are *functional integration* and *self-organization*. Functional integration refers to the interdependence

of biological components that collectively contribute to sustaining an organism or system. Organisms exhibit hierarchical integration, where lower-level biological entities, such as cells and tissues, work together to maintain higher-order functions (McConwell, 2023). Similarly, cells coordinate intracellular activities, while eusocial insect colonies collectively regulate their internal environments (Pepper & Herron, 2008).

Self-organization describes the spontaneous emergence of ordered patterns, structures, or behaviors within biological systems through local interactions among their components, without centralized control (Vidal Arenas, 2024). [INT1] emphasizes that for a biological entity to exhibit functional integration, it must also be capable of self-organization. This involves continuous energy transformations and dynamic interactions that allow biological systems to maintain stability while adapting to change. The self-organizing nature of biological entities contributes to their resilience by enabling them to counter external perturbations through internal regulation.

Adaptation, Temporal Constraints, and Instability

Another essential characteristic of biological entities is their *inherent adaptability* and existence within defined temporal and structural constraints. Unlike non-living objects, which remain unchanged unless acted upon by external forces, biological entities are in a *continuous state of transformation*. "A protein is a consequence of its environment as well as its history.", according to [INT1]. [INT3] similarly notes that biological molecules, such as DNA and proteins, do not inherently possess function, rather, their function emerges when placed in a suitable biological context.

If a biological entity fails to adapt to its environment, it may undergo structural changes, lose functionality, or cease to exist. This can be seen in the death of an organism, the denaturation of a protein, or the degradation of biological macromolecules. While this inherent instability is a defining feature of biological entities, some exhibit periods of dormancy – such as bacterial spores or seeds – where biological activity is temporarily halted until favorable conditions return.

Synthetic Biology Context

The characteristics of biological entities are traditionally defined in relation to naturally occurring biological systems. However, advancements in synthetic biology necessitate a more inclusive definition that encompasses synthetic biological entities to accurately reflect current developments in the field. Synthetic biology is defined as: “The application of science, technology, and engineering to facilitate and accelerate the design, manufacture, and/or modification of genetic materials in living organisms” (Scientific Committee on Health and Environmental Risks et al., 2014). Genetic modification involves altering or editing the genome, which can include the addition of episomal genetic material, such as plasmids or viral particles.

Synthetic biological products are designed and produced using methods aligned with traditional engineering disciplines (Schyfter, 2011). The field aims to engineer useful biological organisms, understand and model biological entities, and uncover their underlying design (ibid.). Products of synthetic biology, excluding bottom-up attempts to synthesize de novo organisms, are entities produced by modifying or mimicking existing organisms, biological processes, or materials. Despite their technological framework, synthetic biology entities are generally considered natural kinds (ibid.).

In conclusion, while the distinctions between biological entities, organisms, and biological individuals remain complex, the outlined characteristics provide a foundational understanding of what could

define a biological entity. Organisms are currently distinguished by their functional autonomy, biological agency, and evolutionary potential, while broader biological entities contribute to these processes through structural and functional integration. Recognizing the diverse nature of biological entities – from molecules to ecosystems – helps refine scientific definitions and philosophical discussions on the nature of life and biological organization. Products from synthetic biology can further complicate our understanding of biological entities. Therefore, it becomes essential to explore the distinctions between naturally occurring and synthetic biological entities, as their differing origins and modes of design raise important questions about classification, function, and biological organization.

4.2 Conceptual Relationship Between Biological Entities and Synthetic Biology

Based on literature reviewed in Sections 3.1 and 3.2, a *naturally occurring* biological entity can be defined as an ordered group of organisms, an organism, a part thereof, or a biological system that has *arisen through evolutionary processes without being designed for a specific function*. These entities are composed of macromolecules synthesized by living organisms and exhibit functional integration, self-organization, and instability. In contrast, a *synthetic* biological entity is an ordered group of organisms, an organism, a part of an organism, or a biologically derived component that has been *intentionally designed, modified, or engineered using synthetic biology techniques*. These entities may be composed of macromolecules synthesized by living organisms or inspired by natural biosynthesis. Importantly, synthetic entities may also exhibit the same core features; functional integration, self-organization, and dynamic behavior.

So, to be classified as a biological entity first of all, a system must possess the following minimum criteria (also see Table 2): (i) composed of biological elements as building blocks; (ii) capable of functional integration; (iii) able to self-organize, and (iv) being in a constant state of change. Figure 3 illustrates the relationship between the terms ‘biological entity’ and ‘synthetic biology’.

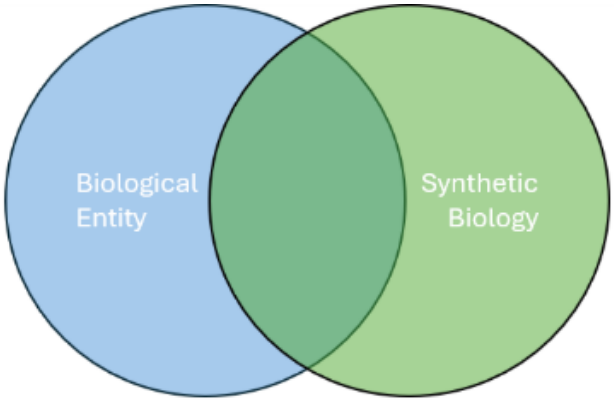

Figure 3: The conceptual relationship between the terms 'biological entity' and 'synthetic biology'.

So, synthetic biological entities, represented as the intersection of biological entities and synthetic biology in Table 3, would meet these criteria. However, synthetic biology entities represent an expansion of the concept of natural biological entities due to the novel combinations they introduce.

Table 3: Differences between the subdivisions for 'biological entity'.

Distinctions in the definition 'biological entity'	
Naturally occurring biological entity	***Synthetic biology entity***
Products of evolutionary processes (origin unknown)	Products of human design (origin known)
Shaped without a specific intent	Designed with an intended function
Biological elements are only synthesized by organisms	Biological elements can be synthesized either chemically or by organisms

Distinctions Between Natural and Synthetic Biological Entities

Naturally occurring biological entities result from millions of years of genetic mutations, phenotypic variation, and evolutionary selection. Their origins are part of a continuous evolutionary process (Deplazes & Huppenbauer, 2009). In contrast, synthetic biology entities are intentionally designed for specific functions, making their origins, to some extent, traceable. Although not direct products of evolution, their development follows biological principles. Despite their artificial origin, synthetic biology entities may develop novel functions beyond their intended design, depending on their construction and application (Schyfter, 2011).

Another distinction concerns the synthesis of biological elements. While natural biological entities synthesize their components internally, synthetic biology entities do not necessarily require biological synthesis. Instead, they may incorporate synthetic biological elements inspired by natural systems. Some synthetic biology entities originate from living organisms, while others are chemically synthesized but replicate biological functions.

Since the term 'organism' falls under the broader category of 'biological entity', synthetic organisms can be considered a subset of synthetic biology entities. As illustrated in Figure 4, synthetic organisms are synthetic biology entities capable of reproduction or genetic exchange, aligning with the definition of an organism adopted by the Ministry of I&W.

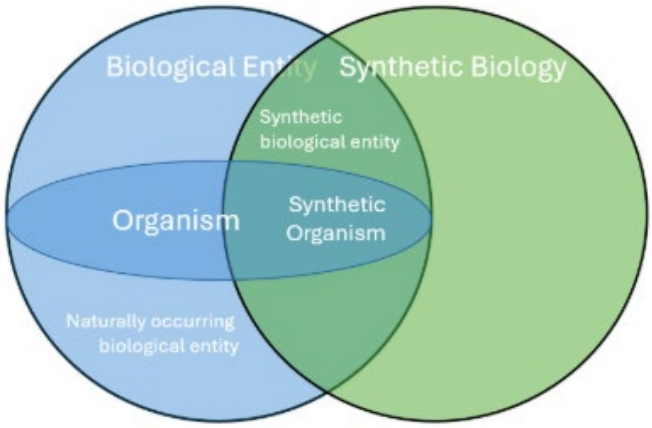

Figure 4: Illustration of the conceptual relationship between the definitions 'biological entity', 'synthetic biology', and 'organism'. The subdivisions in 'biological entity' are provided as 'naturally occurring biological entity', and 'synthetic biological entity', as well as 'organism' and 'synthetic organism'.

Blurred Boundaries Between Natural and Synthetic Entities

While synthetic biology entities share essential characteristics with naturally occurring biological entities, they can also differ in origin, synthesis, and intended function. Discussions in the focus group (Chapter 5) also gave rise to questions on the effectiveness, and successful practical implementation of such distinction. One of the perceived primary limitations of such distinction is the difficulty in maintaining the boundary between ‘natural’ and ‘synthetic’. As illustrated by the presented borderline cases (Chapter 6), advances in genetic engineering have enabled the creation of organisms that combine natural components in novel ways or mimic natural evolution under laboratory control. So, many ‘entities’ exist on a continuum between being ‘fully’ natural and ‘fully’ synthetic. For instance, a yeast strain engineered with synthetic chromosomes may functionally resemble its wild-type counterpart but differ substantially in origin and design. Or, synthetic cells, built from the same macromolecular components as natural cells, could eventually gain the ability to self-replicate, repair, and mutate, paralleling the behavior of natural organisms. If evolutionary processes modify synthetic entities over time, should their original artificial classification remain (Deplazes & Huppenbauer, 2009)? In such cases, a binary classification could add more ambiguity than it would clarify, particularly as synthetic biology entities could evolve beyond their original design.

5 LoT 2: Towards an interaction-based perspective on Biological Entity

The challenges outlined in distinguishing between naturally occurring and synthetic biological entities underscore the limitations of binary classifications, especially as many entities occupy a continuum shaped by both natural and engineered influences. As synthetic biology advances, there is a growing need for more nuanced criteria that account not only for an entity's origin or composition but *also* for its (potential) behavior and context. LoT 2 meets this need by offering an interaction-based, forward-looking perspective. Developed with expert input from a focus group, this set of criteria emphasizes an entity's potential to act within its environmental context, its capacity to interact with living systems, and the extent to which it can be controlled or reversed. By shifting the focus from static definitions to dynamic potential and impact, LoT 2 can provide a more flexible and context-sensitive approach to evaluating biological entities in light of ongoing scientific and technological developments.

From 'static' to 'potential to'
The focus group highlighted the inadequacy of the current legal definition of a 'biological entity' in light of advances in synthetic biology. Participants acknowledged the four core characteristics (LoT 1) which were identified through the philosophical and biological analysis; *biological composition, functional integration, self-organization*, and being in a *constant state of change*, or dynamic behavior. However, while the group regarded these characteristics as foundational, these were also deemed insufficient to cover *emerging* synthetic constructs. Discussions emphasized the importance of *potential function*, the *environmental context*, and *human design* and *control* for determining whether something could or should classify as a biological entity. The group pointed out grey-area cases like self-replicating RNA-lipid particles, mirror proteins, and protocells.

Lipid nanoparticles carrying self-replicating RNA (srRNALNPs) were provided as a case (number 2, Chapter 6) by the Ministry of I&W, and were also acknowledged as a borderline example by participants of the focus group. It was confirmed that these particles blur the line between inert delivery systems and biologically active agents due to their capacity for replication once inside host cells. With regard to the four characteristics of LoT 1, srRNA-LNPs would only partially meet the four proposed criteria for a biological entity:

1. *Biological composition*: the lipid nanoparticle itself is typically synthetic or semi-synthetic, often designed for stability and delivery rather than being biologically derived. Although the encapsulated RNA is a biological molecule, the overall construct was seen as lacking coherent biological makeup, especially if the lipid components do not resemble natural membranes.
2. *Functional integration*: the srRNA and the lipid carrier are not intrinsically interdependent. The lipid merely serves as a passive delivery vehicle and does not interact functionally with the RNA beyond protecting it during transport. This weakens the case for integrated functionality, which is an indication of natural biological systems.
3. *Self-organization*: the system does not self-assemble into a stable structure in situ; rather, it is assembled artificially in the lab. Unlike cells that spontaneously organize their internal structures, these particles require external input to form and function, which undermines their claim to self-organization.

4. *Constant state of change*: while the RNA component may replicate once inside a host cell, the LNP as a whole does not exhibit autonomous dynamics. Its activity is entirely dependent on cellular machinery, and outside the cell it is inert, showing no metabolism, growth, or interaction with its environment.

In short, the group noted that although srRNA-LNPs *might produce biological effects*, their own properties do not sufficiently align with the initially established criteria for being called a biological entity.

Another discussed borderline case was mirror-image proteins, composed of D-amino acids, which resist natural degradation and do not interact with biological systems in conventional ways, raising concerns about environmental persistence rather than replication. The group also examined protocells, synthetic vesicles capable of ribozyme-based replication without DNA, as well as the minimal bacterium, a stripped-down bacterial cell engineered to retain only the essential genes required for 'life'. Although this minimal cell can replicate, it relies heavily on a controlled environment and lacks many metabolic capabilities, prompting debate over its 'autonomy' as a biological system. These examples illustrate that classical criteria like replication or genetic transfer are no longer fitting, as well as the four proposed characteristics from the literature review that would define a biological entity. In response, the focus group led to the establishment of three alternative criteria – *context dependence*, *potential for interaction*, and un*controllability/irreversibility* – as essential complements to the current defined features of biological entities.

Participants emphasized that the status and behavior of a biological entity are rarely absolute. Instead, they are heavily dependent on their respective *environmental context*. An entity might appear inert in isolation but become active or hazardous in a particular ecological or cellular setting. For example, a self-replicating RNA particle might be harmless outside a cell, but once internalized, it hijacks host machinery and becomes functionally 'alive'. The group argued that biological definitions should account for latent or conditional properties, not just the static, present-state behavior of an entity. This perspective encourages a dynamic view of entities, assessing what they can become or do in real-world settings, rather than focusing only on what they are under laboratory conditions.

Following upon, rather than requiring current, observable interaction, the group proposed that '*potential' to interact meaningfully* with biological systems should be a defining feature. This includes the ability to enter cells, interfere with genetic pathways, or alter ecological dynamics. For instance, synthetic proteins that mimic natural hormones or inhibitors may not replicate or evolve, but they can significantly disrupt physiological systems. The idea here is that biological risk and relevance begin at the threshold of meaningful interaction, even in the absence of classical biological traits like replication or metabolism. This criterion shifts focus from structure to functional implications; does the entity have the capacity to engage with living systems in a biologically consequential way?

Lastly, the group discussed the illusion of control that often accompanies synthetic biology. In laboratory settings, many constructs (e.g., gene drives, minimal cells) seem well-contained, or programmable. However, once released into complex environments, predictability and control can rapidly decrease. A key example mentioned was synthetic chassis organisms: while engineered to require specific nutrients or triggers to survive, mutations or ecological interactions could quickly undo that control. In conclusion, the criterion here should not be 'is it controllable under ideal conditions?', but rather 'can it remain controllable or reversible in realistic contexts'?

In Summary

From these discussions, three critical supplementary criteria emerged for LoT 1: *context dependence*, *potential for interaction*, and un*controllability* or ir*reversibility* (See Table 4). First, participants stressed that the biological status of an entity often depends on its environmental context. An entity inert in one setting may become biologically active or hazardous in another, suggesting that latent or conditional properties should be integral to the set of criteria in LoT 2. Second, the potential for interaction with living systems, rather than current observable interaction, was identified as a more meaningful indicator of biological relevance. This includes the capacity to enter cells, influence genetic processes, or disrupt ecological balances – even in the absence of traditional traits like replication or metabolism. Third, the group questioned the *sustainability of control* over synthetic systems. Constructs perceived as controllable under laboratory conditions could become unpredictable or irreversible when introduced into real-world environments, as illustrated by examples such as gene drives and synthetic chassis organisms.

Together, these findings lead to a more dynamic and anticipatory perspective for classifying biological entities – one that moves beyond static features toward a functional, relational, and context-sensitive approach. These insights laid the groundwork for the development of LoT 2, an interaction-based, forward-looking framework that better captures the complexities introduced by synthetic biology.

Table 4: An interaction-based, forward-looking perspective, and characteristics to define a 'biological entity'.

LoT 2: Biological Entity Characteristics		
Environmental Context	Potential to Interact	Uncontrollability/ Irreversibility

6 Case Studies

To explore the applicability and interpretive value of the two developed LoTs, we apply them to three borderline cases, provided by the Ministry of I&W. This application is intended as an exploratory exercise aimed at assessing the extent to which the LoTs and their respective characteristics meaningfully align with the complexities presented by the borderline cases. Through this analysis, we aim to gain insights into the practical relevance of the LoTs, identify areas of ambiguity or tension, and highlight potential points of discussion for future refinement.

The first LoT (Chapter 4) was developed through analysis of the philosophical and biological context (Section 3.2), focusing on assessment on foundational components, like composition of biological elements, functional autonomy, agency, and structural integration. This LoT is based on how a biological entity is *currently* defined in literature and thus reflects the defined characteristics as they are now. The second LoT, being complementary to LoT 1, was developed by means of the focus group (Chapter 5), and focuses on the specific environmental context and an entity's 'potential to act', thereby acknowledging and anticipating *future* developments in the field of synthetic biology. Table 5 below provides an overview of both LoTs.

Table 5: Overview of two lines of thought (LoT). LoT 1 is a composition-based perspective and entails 4 characteristics for a biological entity based on current usage of the notion. LoT 2 is an interaction-based, forward-looking perspective which focuses on anticipating future developments in synthetic biology, building upon LoT 1.

Characteristics based on currently used definition of biological entity **LoT 1: Focus on foundational components**	**Characteristics anticipating developments in synthetic biology** **LoT 2: Focus on potential to act**
Biological Composition	Environmental Context
Functional Integration	Potential to Interact
Self-organization	Uncontrollability/ Irreversibility
Constant State of Change	

6.1 Case 1: Infectious Ribonucleic Acid (RNA)

RNA is a biological molecule with biological characteristics but is not classified as an organism (Scientific Committee on Health and Environmental Risks et al., 2014). This case examines the positive-stranded RNA poliovirus. While the virus contains all the genetic properties necessary to produce infectious particles, it lacks its own RNA polymerase and must first be translated within the host cell (Ahlquist et al., 2003). Viruses have complex life cycles, with positive-strand RNA playing a crucial role (Hogle, 2002). The RNA genome evolves through mutations, enhancing adaptability (Savolainen-Kopra & Blomqvist, 2010), yet remains unstable due to cellular RNA degradation pathways (Ahlquist et al., 2003).

LoT 1: Focus on foundational components

[INT3] supports classifying the case on infectious RNA as a biological entity, as it functions within a cellular context (biological composition). While viral genomes act as templates for replication and translation, they do not encapsidate their own RNA polymerase. Instead, translation must occur first

to produce the polymerase enzyme, enabling genome replication and viral assembly (functional integration) (Ahlquist et al., 2003).

Poliovirus RNA, like other biological entities, replicates inside the host (Racaniello, 2006) and can also be synthetically constructed (Wimmer & Paul, 2010). Functionally, the RNA genome integrates with the host's cellular machinery, facilitating its own survival through translation and replication (Hogle, 2002). Its self-organizing properties are evident in its cytoplasmic translation and encapsidation, which is tightly linked to replication (Hogle, 2002).

In conclusion, based on the characteristics defined in LoT 1, infectious RNA would be considered a biological entity.

LoT 2: Focus on potential to act

The biological activity of poliovirus RNA is highly context-dependent. In extracellular environments, the naked RNA is inert and only becomes biologically functional when delivered into a permissive host cell, where cellular ribosomes translate it into viral proteins. These include the viral RNA-dependent RNA polymerase, which is necessary for genome replication (Hogle, 2002). Thus, its biological activity is latent and triggered only in the right intracellular environment (environmental context). Once inside a host, the RNA genome immediately engages in meaningful biological interaction. It not only initiates protein synthesis but also orchestrates the formation of replication complexes through the reorganization of host cell membranes (Ahlquist et al., 2003).

The genome is also subject to high mutation rates, which facilitate rapid evolution and host adaptability (Savolainen-Kopra & Blomqvist, 2010). These interactions have substantial physiological consequences for the host and demonstrate the RNA's functional agency within biological systems – meeting the characteristic on potential to interact. The third criterion (uncontrollability) is also fulfilled. The RNA genome actively engages with the host's cellular systems to ensure its own persistence by initiating translation and driving replication (Hogle, 2002). Its capacity for self-organization is demonstrated by the way translation and encapsidation occur within the cytoplasm in direct coordination with the replication process. These factors underline the issue of uncontrollability/irreversibility of poliovirus RNA outside secure, regulated environments.

In conclusion, though not an organism in the taxonomic sense, its capacity to activate contextually, interact profoundly with living systems, and resist long-term control in practice, supports its classification as a biological entity in line with LoT 2.

6.2 Case 2: Lipid Nanoparticles (LNPs) with Self-Replicating RNA (srRNA)

LNPs are lipid vesicles widely used for drug and nucleic acid delivery (Eygeris et al., 2021). Self-replicating RNA, derived from positive-strand RNA viruses, is encapsulated within LNPs for enhanced stability and intracellular delivery (Tews & Meyers, 2016). LNPs are lipid vesicles used for delivering small-molecule drugs and nucleic acids, including self-replicating RNA (srRNA) derived from positive-strand RNA viruses (Eygeris et al., 2021; Tews & Meyers, 2016). LNPs have been successfully applied in mRNA vaccines, such as for COVID-19, and hold promise for cancer immunotherapy, protein replacement, and gene editing (Eygeris et al., 2021). LNPs protect unstable mRNA from rapid degradation, facilitating intracellular delivery (Eygeris et al., 2021).

This case is an illustrative for a borderline case on when something can or cannot be deemed a biological entity. Based on the expert interviews, this case would be considered a biological entity according to the criteria developed in LoT 1. However, during the focus group, this particular case was also discussed, and it was concluded that this case would only *partially* meet the requirements of LoT 1, thus not being considered a biological entity.

LoT 1: Focus on foundational components

[INT2] distinguishes between LNPs with srRNA and isolated LNPs, noting that while lipid membranes form through thermodynamic forces, the complete system carries information that initiates biological processes. Similarly, [INT3] compares this to infectious RNA, highlighting that LNPs interact with the cell membrane while delivering their content. Though empty LNPs can be synthesized (Simonsen, 2022), in this case, positively charged LNPs form electrostatic bonds with negatively charged RNA, stabilizing the nanoparticles. Without RNA, LNPs would be unstable, making their classification as a biological entity in isolation untenable. LNPs with srRNA exhibit key characteristics of biological entities. They self-organize during synthesis, assembling into nanostructures through intermolecular interactions (Eygeris et al., 2021). Though chemically synthesized, their composition is inspired by biological entities such as exosomes (Zhang et al., 2019) and liposomes (News-Medical, 2019). LNPs also integrate functionally with their environment, interacting with cellular membranes and incorporating into cellular processes upon entry (Eygeris et al., 2021). Additionally, LNPs undergo structural changes in response to environmental triggers and are prone to aggregation, which can lead to RNA loss (Eygeris et al., 2021).

Since LNPs with srRNA meet the criteria for biological entities, they *should* be classified as biological entities due to their designed function and chemical synthesis. [INT1] underscores that while LNPs biologically interact with cells, they are not naturally produced by them, marking a key distinction.

On the other hand, the focus group (Chapter 5) acknowledged that while self-replicating RNA (srRNA) lipid nanoparticles (LNPs) can trigger biological effects once delivered into a host, their inherent properties do not meet the core criteria for being classified as biological entities.

First, in terms of biological composition, the LNP itself is typically synthetic or semi-synthetic, designed primarily for stability and delivery efficiency. Although the encapsulated RNA is a biological molecule, the overall construct lacks the coherent biological makeup expected of natural biological systems, particularly when the lipid components do not resemble natural cellular membranes. Second, the functional integration between the RNA and the lipid carrier is minimal. The lipid nanoparticle serves mainly as a protective delivery vehicle and does not interact with or influence the RNA functionally beyond shielding it during transport. This limited interdependence contrasts with the tightly integrated systems found in natural biological entities. Third, the construct does not exhibit self-organization. Unlike living cells, which spontaneously assemble and maintain internal order, srRNA–LNP complexes are entirely manufactured through external laboratory processes. They do not self-assemble or self-regulate once introduced into biological environments, which weakens their claim to biological status. Finally, regarding dynamic behavior, the LNP itself remains inert outside the host cell. While the RNA component may replicate once inside, the LNP as a whole does not show autonomous behavior such as metabolism, growth, or environmental interaction. Its activity is fully dependent on host cellular machinery.

In sum, although srRNA–LNPs may produce significant biological outcomes, their structure, function, and origin do not align closely enough with the foundational criteria to be considered biological entities in their own right.

LoT 2: Focus on potential to act

LNP–srRNA constructs are active inside cells. The srRNA stays inactive until the LNP delivers it into a host cell. There, the RNA is used by the cell's ribosomes to produce proteins, including one that helps the RNA copy itself (Tews & Meyers, 2016). Outside the cell, the RNA is unstable and easily broken down. This shows they are clearly context-dependent (Eygeris et al., 2021), and do not have the potential to interact outside of their cell environment. However, once inside the body, the RNA starts making proteins and boosts immune responses. The LNP itself helps by acting like a built-in adjuvant: it attracts and activates immune cells (Eygeris et al., 2021), showing strong interaction with living systems.

LNP-srRNA constructs are hard to control as the RNA can keep producing proteins for days or weeks, unlike normal mRNA, which fades quickly (Vogel et al., 2018). Biodistribution studies show that LNP components and encapsulated RNA can migrate beyond the site of administration. For instance, RNA and/or lipid components have been detected in blood plasma and breast milk following mRNA vaccination (Röltgen et al., 2022). Additionally, the self-replicating nature of the RNA significantly extends its duration of expression. Compared to conventional mRNA, which degrades rapidly, srRNA can drive sustained antigen production over several days or even weeks, reducing the ability to fine-tune or reverse its effects post-administration (Tews & Meyers, 2016). Also, as the full sequences of viral replicons used in srRNAare public, they can be recreated outside labs, thus outside controlled settings. The European Medicines Agency (EMA) does not currently classify srRNA–LNP vaccines as genetically modified products[29], meaning they bypass environmental risk assessment before market approval, despite their self-amplifying properties. In response, the Dutch COGEM and German ZKBS issued a joint open letter urging the European Commission to reconsider this position[30].

In conclusion, LNP–srRNA constructs meet all three conditions: they become active once in a host cell, they strongly affect living systems, and they're difficult to control outside contained, controlled environment. So, while not considered living organisms, they 'act' according to the three established criteria and can therefore be deemed a biological entity.

6.3 Case 3: Minicells for Double-Stranded RNA (dsRNA)

Minicells, aberrant products of bacterial cell division, lack chromosomes but retain functional cellular components (Farley et al., 2016). Used in RNA interference (RNAi)-based fungal disease control, they encapsulate dsRNA that silences essential pathogenic genes (Islam et al., 2021). Minicells arise from aberrant cell division due to polar Z-ring placement. First discovered in *Escherichia coli* (E. coli), they have since been observed in other rod-shaped bacteria. Minicells contain membranes, ribosomes, RNA, proteins, and often plasmids but lack chromosomes, preventing division or growth while sustaining cellular functions like ATP synthesis, mRNA translation, and plasmid replication (Farley et al., 2016). They are used for producing and delivering dsRNA, which triggers RNA

[29] https://www.ema.europa.eu/en/documents/assessment-report/kostaive-epar-public-assessment-report_en.pdf
[30] https://zkbs-online.de/en/service/commentaries/open-letter-cogem-zkbs-era-samrnas

interference (RNAi) to silence fungal pathogenicity genes, inhibiting *Botryotinia fuckeliana* growth for 12 days in greenhouse conditions (Islam et al., 2021).

LoT 1: Focus on foundational components

Minicells are typically derived from mutant *E. coli* strains with deletions in cell-cycle genes, intentionally modified yet still considered organisms (Islam et al., 2021). As they originate from a synthetic organism and contain biological elements like RNA and proteins, they meet the criteria for biological entities. Their formation, despite incomplete division, demonstrates self-organization, and their ability to sustain essential cellular functions allows survival. [INT3] describes them as organelle-like structures existing outside cells, capable of cellular processes. Their functional integration is evident in plasmid DNA transfer, despite lacking a genome.

Minicells also exhibit instability; when delivering dsRNA to a target cell, they are internalized, release their content, and are degraded by the host. Lacking chromosomal DNA, their ability to self-repair depends on available RNA and plasmids. [INT1] compares them to red blood cells, calling them "physiologically living but genetically dead" due to their inability to reproduce. As *E. coli* minicell-producing strains are considered synthetic microorganisms, minicells themselves are classified as biological entities. Their ability to transfer plasmid DNA further qualifies them as synthetic organism.

LoT 2: Focus on potential to act

Minicells display latent biological activity in specific contexts. Though incapable of growth or division due to the absence of chromosomes, they maintain key biological processes such as ATP production, protein synthesis, and plasmid replication (Farley et al., 2016). These functions only occur in suitable physiological conditions, such as appropriate temperature, pH, and access to nutrients or cofactors. Therefore, this demonstrates that their functional capacity is only under defined environmental and biological conditions and therefore has no potential to interact with its environment outside of these conditioned settings. However, key biological processes continue regardless of controlled settings – therefore the condition of environmental context is only partially met – ‘it has the potential to‘.

Furthermore, minicells can affect other organisms. When used to deliver dsRNA, they can stop the growth of plant pathogens like *Botryotinia fuckeliana* by turning off essential fungal genes. They also still have biological machinery and can synthesize proteins or carry out basic processes inside cells (Islam et al., 2021). So, they interact in a meaningful way with living systems and meet the second criterion of potential to interact. Lastly, minicells cannot divide or evolve and thus have controllability, being a major safety advantage. However, minicells are not inert. They can remain active for several days, continuing to function inside their environment, although minicells do have higher controllability than ‘full’ cells. Therefore, also the condition of uncontrollability/irreversibility is partially met.

In conclusion, as the field of synthetic biology continues to advance, the definition of ‘biological entity’ must evolve to accommodate synthetic constructs. While functional integration and self-organization are key characteristics of biological entities, they are not exclusive to biology. Machines, such as cars, exhibit functional integration, while non-biological systems, such as freezing water, also demonstrate form of self-organization even though induced by external forces.

While LoT 2 is a complementary approach to LoT 1, this second set of defined criteria provides an anticipatory approach to emerging applications and shifts the focus to a dynamic view of entities, their functional implications, and whether it can remain controllable or reversible in realistic contexts. Table

6 below provides an overview of the three examined case studies, the extent these meet the criteria in line with both LoT, and whether or not each case can be regarded as a biological entity.

Table 6: overview of all three cases, and whether or not these are classified as a biological entity according to the two developed LoTs.

Biological Entity	**Case 1 Infectious RNA**	**Case 2 LNPs with srRNA**	**Case 3 Minicells**
LoT 1	Yes, all criteria met.	*Undetermined*	Yes, all criteria met.
LoT 2	Yes, all criteria met.	Yes, all criteria met.	*Undetermined*

It should be noted that it is undecided whether or not case 2 could be defined as a biological entity based on the characteristics of LoT 1. However, according to criteria from LoT 2, LNPs with srRNA should be regarded as biological entities. For case 3, the minicells, these would be classified as a biological entity according to LoT 1. However, the criteria for LoT 2 were only partially met. For instance, the system functions only under specific physiological conditions, its ability to interact with the environment is limited outside of controlled settings, and minicells cannot divide or evolve, which enhances their controllability. Whether these partially met criteria are sufficient to classify the system as a biological entity remains undetermined. Such a determination would depend on regulatory and political decisions and is therefore beyond the scope of this study – for example, is having only the *potential to* interact enough? Or would e.g. a minicell not be considered a biological entity as long as it remains in limited, controlled settings in which it cannot function?

7 Conclusion & Recommendations

This study set out to explore how the concept of a *biological entity* can be meaningfully defined from biological, philosophical, and legal perspectives in light of emerging developments in synthetic biology. The goal was to develop a more robust set of characteristics for classification and regulation, especially in light of increasingly blurred boundaries between natural and synthetic entities. Drawing from extensive literature review, expert interviews, and focus group insights, this work proposes a reconceptualization of what constitutes a biological entity and outlines the implications for regulatory systems.

Limitations of Current Definition

Our findings reveal limitations in current legal and scientific definitions of biological entities. Definitions (implicit and explicit) used in present regulatory frameworks seem to rely heavily on origin (natural vs. synthetic) or static characteristics (e.g., replication or genetic transfer) to determine whether something qualifies as an organism, and thus a biological entity. However, analysis of the legal context, including the ECJ's decision in Bablok and Others v. Freistaat Bayern, showed that regulation should not put emphasis on origin alone as this would not be a reliable indicator of potential (harmful) ecological impact. What matters more is how a biological entity interacts with its environment, for instance its potential for persistence, dispersal, gene transfer, or unintended ecological disruption. A naturally occurring (micro)organism introduced into a new habitat could just be as ecologically disruptive as a fully synthetic one. Similarly, a genetically modified organism used strictly in a contained industrial process may pose minimal environmental risk, despite its artificial origin. Thus, potential risk (and thus regulatory oversight for safety) would be better assessed through function, context, and exposure rather than origin. In addition, proving synthetic origin for enforcement purposes can be technically challenging or impossible once the entity is released or propagated in the environment.

Arriving at a Shared Definition

Initially, four criteria for a biological entity were derived from the philosophical and biological literature review and expert interviews (LoT 1). These were: (i) composed of biological elements as building blocks; (ii) capable of functional integration; (iii) able to self-organize, and (iv) being in a constant state of change. However, further analysis revealed that these criteria do not sufficiently fit advancements in synthetic biology and would thus not contribute to resolving borderline cases. For instance, srRNA-LNPs *might produce biological effects*, their own properties do not sufficiently align with the initially established criteria for being called a biological entity, and new synthetic constructs often combine natural components in novel ways or mimic natural evolution under laboratory control.

For these reasons, we propose a shift toward a risk-based classification system that focuses on 'behavior' and potential interactions with environments (i.e. LoT 2, Chapter 5). The established criteria for LoT 2 were: (i) context dependence, ii) potential for interaction, and iii) uncontrollability/ irreversibility. The status and behavior of a biological entity is *context-dependent* rather than absolute. For instance, an entity may appear inert in isolation but become active or hazardous in specific environments. A srRNA particle may be regarded harmless outside of a (host)cell, but functionally 'alive' when internalized. This illustrates the importance of considering latent or conditional properties, and not present-state behavior. Along that line, another defining criterium for a biological entity should

be an entity's *potential to interact* meaningfully with other systems, e.g., entering cells, gene expression or disruption of ecological processes. Even entities that lack replication or metabolism like synthetic proteins imitating hormones, can have significant biological effects. Lastly, *control* may be misleading in synthetic biology. Though constructs may appear controllable under contained settings, natural evolution can be mimicked too, and real-world environments can also introduce unpredictable interactions. Additionally, due to this, engineered safety mechanisms can fail too. Therefore, a more robust definition of a biological entity should not account for whether and entity *can* be controlled, but for whether that control is sustainable in realistic, real-world contexts and environments.

Such approach could be more adaptive, scientifically grounded, and responsive to emerging technologies. Also, it could support proactive, resilient governance as this approach emphasizes potential 'behavior' compared to more abstract categories like origin and the type of components an entity would be made of. Ultimately, this could enable more effective and balanced oversight, fostering responsible research and innovation in synthetic biology. However, a key regulatory challenge would be that synthetic biology entities that undergo evolutionary processes, cannot always be fully predicted or contained (Deplazes & Huppenbauer, 2009). Further research would be needed to assess the role of evolutionary principles in synthetic biology and to determine the extent to which this would infringe with the criteria of 'uncontrollability/irreversibility'.

A Reframed Definition

Based on our analysis, we propose the following definition of a biological entity: "*any material construct, system or component, regardless of origin or current activity, that has the actual or potential capacity to interact meaningfully with living systems or facilitate biological processes in an ecologically or physiologically relevant context*".

Hereby, the following connotations are made:

- "any material construct, system or component" thus includes 'traditional' organisms, viruses, genetic constructs, synthetic (proto)cells, RNA particles, engineered proteins and more.
- "regardless of origin or current activity" avoids making a distinction between natural and synthetic, and accounts for some entities being inert under laboratory conditions but can become active under specific conditions.
- "to interact meaningfully with living systems or facilitate biological processes" shifts the focus from structural traits like metabolism or replication, to functional relevance.
- "in an ecologically or physiologically relevant context" ensures that interaction potential is not considered in the abstract, but in relation to real-world conditions.

Such a definition and its associated criteria could allow regulators to better anticipate emergent behaviors and novel risks, particularly from synthetic constructs designed to be more modular, passive, or 'non-living' in traditional terms. While the definition is intentionally broad, it also provides conceptual boundaries. For instance, although molecules such as water and organic compounds like PFAS contain similar atomic elements as biological macromolecules, they are not structured through biological processes nor do they independently participate in biological functions – they may influence processes, but do not facilitate these. While essential for life, they act as passive contributors rather than forming active biological entities. Meanwhile, the proposed definition and connotations help clarify such distinctions by focusing on meaningful interaction within ecologically or physiologically relevant contexts, ensuring that only entities with functional biological relevance fall within regulatory scope.

Regulatory Implications

Analysis of the term biological entity from a legal perspective revealed that the term serves as a foundational legal concept in biotechnology regulation, where it is broadly defined as any living system capable of replication or transferring genetic material. However, this terminology can no longer provide an accurate basis for regulation because of vast scientific developments in the field. Additionally, keeping up with such developments turns out to be even more complicated due to the fundamental difference between legal and biological logic. For example, while biologists generally do not consider viruses to be (living) organisms due to their dependence on a host cell, legislation does classify viruses as such (Directive 2009/41/EC). This choice is not necessarily wrong, but it is clear that it serves a different purpose: legal definitions are often oriented toward risk and controllability, rather than scientific accuracy.

Our reframed definition and connotations of biological entity addresses this challenge. These criteria would allow for a more flexible assessment based on what an entity does or can do in specific environments, rather than fixed properties in isolation. Such an approach would also meet another recurring point of tension, related to the evaluative timeline in the current permitting system. Under the prevailing model, it is first determined whether something falls within the scope of the law – can 'it' be defined an organism – and only then is it assessed for safety. The interviews and analysis revealed that reversing this process might be more effective. That is, if the definition of a biological entity would become more risk oriented to begin with, thus assessing on 'potential to' and thus assessment for safety. Also, an interaction-based, forward-looking approach would align with the idea of 'resilient regulation', which is a proposed legal framework that can adapt to rapid scientific and technological changes without losing legal clarity or compromising public safety[31].

Furthermore, the current regulatory system has adopted a function-based approach, which is also used across the EU, Australia, Canada, and historically in the U.S., and ensures flexible oversight of genetically modified and synthetic organisms. While effective for regulating traditional (GM) organisms, it faces limitations with non-replicative or cell-free technologies emerging from synthetic biology. There are several alternatives: composition-based (what is an entity made of?), taxonomic-based (origin of the organism), and interaction-based (how an entity behaves in, or affects biological systems).

The composition-based approach is, for instance, used by the U.S. Food and Drug administration for regulating gene therapies[32] which is based on the components, e.g. viral vectors or gene-editing tools. Also, the OECD Working Party on Biotechnology uses this form to discuss regulation of synthetic constructs by their molecular design[33]. A taxonomic-based approach is implicitly used by the Convention on Biological Diversity[34] when referring to biological diversity, thereby assuming relevance to organisms with recognized taxonomic groups. This approach features also in the Nagoya Protocol (EU Regulation 511/2014[35]) which is primarily applied to naturally occurring organisms and their genetic resources and excludes some synthetic organisms if their origin can't be traced back taxonomically. An interaction-based approach is used in the Cartagena Protocol on

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[31] See for example Poort & Kortleven, 2025 via https://pure.eur.nl/en/publications/grijze-gebieden-in-de-regulering-van-groene-en-rode-biotechnologi
[32] https://www.fda.gov/media/106369
[33] https://www.oecd.org/content/dam/oecd/en/publications/reports/2014/06/emerging-policy-issues-in-synthetic-biology_g1g3c829/9789264208421-en.pdf
[34] https://www.cbd.int/convention/articles?a=cbd-02
[35] https://eur-lex.europa.eu/eli/reg/2014/511/oj/eng

Biosafety[36] – using 'living modified organisms' – in which the risk assessment guidelines focus on interactions with the environment such as toxicity or ecological fitness, regardless of how the organism was made. Further studies could be devoted to seeing if one of these alternative approaches could make respective regulation future-proof and whether, and to what extent these could also add ambiguity or regulatory burden.

Suggestions for Future Research

Reframing the Concept of 'Biological' in Regulation

Future research could explore whether the continued use of the term *biological* in classifying entities is conceptually necessary or whether 'only' *entity* might be more appropriate given developments in synthetic biology. As constructs increasingly blur the lines between biological, chemical, and engineered systems, the distinction implied by *biological* may become less meaningful or even limiting. This line of inquiry could also examine the implications of such a shift for coherence across regulatory frameworks such as REACH and GMO legislation, and whether partial or functional integration of these regimes might improve regulatory consistency for hybrid or borderline entities.

Defining the Thresholds of 'Potential to' Interact

Further research can clarify the boundary conditions for regulatory relevance under a 'potential to interact' definition. For example, should synthetic constructs not be classified as biological entity as long as they are strictly contained, and only come under regulation upon release or environmental exposure? Current developments such as the creation of synthetic cells[37] and mirror-life[38], could serve as provocative test cases to examine how far potentiality should extend as a regulatory trigger.

Assessing Acceptable Levels of Containment and Safety

Building upon the previous recommendation for future research, there is also a need to examine how different levels of biological containment are currently interpreted as 'safe enough' in regulatory practice, and whether these thresholds remain adequate in light of emerging technologies. This includes evaluating the residual risks associated with contained use and how regulators can balance precaution with innovation.

Integrating Biosecurity into Biosafety Frameworks

As synthetic biology advances, e.g. creating synthetic cells and mirror-life, dual-use concerns grow more pressing. Future studies could explore how biosecurity considerations such as misuse or malicious reapplication can be proactively addressed within existing biosafety legislation, and early in the research and development cycle, potentially making it a formal component of risk governance.

Designing Resilient Legal Frameworks

To ensure regulatory systems remain effective in the face of rapid scientific change, more research could be devoted to *resilient legislation*. This includes analyzing how legal frameworks can remain adaptable without sacrificing clarity or enforceability.

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[36] Annex III of the Cartagena Protocol on Biosafety: Risk Assessment via https://bch.cbd.int/protocol/text/article.shtml?a=cpb-43

[37] For instance, the 'Building a Synthetic Cell (BaSyc) project via https://www.basyc.nl/ or EVOLF – Evolving life from non-life via https://www.evolf.life/.

[38] https://news.stanford.edu/stories/2024/12/potential-risks-of-mirror-life#:~:text=In%20natural%20living%20organisms%2C%20DNA,to%20as%20%E2%80%9Cmirror%20life.%E2%80%9D

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Appendix A – Natural Entity

Besides *biological* entity, the notion of *natural entity* has more recently entered legal discourse. While these terms may appear to have similarities at first glance, they serve distinct functions in legal texts around the world – biological entity being grounded in scientific classification, whereas natural entity is shaped by normative and regulatory assumptions about nature.

The term *natural entity* has gained attention in recent years, particularly in the context of environmental personhood, where ecosystems or landscapes are increasingly recognized as potential subjects of legal rights. Exploring the concept of *natural entity* alongside *biological entity* offers a broader and more interdisciplinary perspective, contributing to a better understanding of how legal systems distinguish and relate biological and other natural elements. This is particularly relevant in disciplines such as law and philosophy, where the terms may carry different connotations or intersect in complex ways. Moreover, examining the concept of *natural entity* suggests that legal frameworks are not solely grounded in biological facts, but may also reflect cultural, ethical, or political interpretations of nature. While such an analysis may not directly resolve definitional challenges related to biological entity, it can encourage reflection on how regulatory approaches might evolve, shifting from origin-based distinctions (natural vs. artificial) toward more functional, risk-based, or relational models. In this way, it can provide insights into how legal categories influence and shape opportunities for innovation, responsibility, and governance in the field of biotechnology.

Natural Entity in European Law

The term natural entity represents a major shift in legal thinking, moving from managing living organisms to recognizing ecosystems as legal persons. The most notable example is Spain's Law 19/2022[39] on the Mar Menor lagoon; the first European statute to grant full legal personality to an ecosystem. According to article 1, the lagoon is declared a 'legal subject' and is subsequently referred to as a natural entity. This grants legal personality to the Mar Menor lagoon and makes it possible for the ecosystem itself to have standing in court and for its rights to be defended by appointed guardians. This legal innovation marks a departure from the traditional human-centered environmental laws that focus on regulating pollution or resource use. Instead, it frames the ecosystem as a subject with inherent rights. The concept has been heavily influenced by Indigenous legal traditions (mostly from outside of Europe) and recent ecological movements are seeking to transform legal systems in response to planetary crises. Illustrative is a 'soft law' European Parliament A9-0087/2023[40] and the associated think tank study 'Can Nature Get it Right?'[41] – a call on the Commission to explore legal recognition of natural entities as right holders, showing political momentum.

In the Netherlands, currently, a natural entity only legally refers to a human being, as opposed to a legal entity which would be a company or an organization with its own legal rights. In the past, some

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[39] Mar Menor Act via https://ecojurisprudence.org/wp-content/uploads/2022/02/Spain-Rights-of-Mar-Menor-Law.pdf (in Spanish) or https://ecojurisprudence.org/initiatives/proposed-law-for-recognition-of-legal-personality-to-the-laguna-del-mar-menor-and-its-basin/ (in English)

[40] https://www.europarl.europa.eu/doceo/document/A-9-2023-0087_EN.html#:~:text=This%20Directive%20aims%20to%20provide, relevant%20Union%20environmental%20sectoral%20legislation.

[41] https://www.europarl.europa.eu/thinktank/en/document/IPOL_STU(2021)689328

research in Dutch contexts has been conducted on the term natural entity and its relation to 'nature', e.g., see Verhoog, Matze, Van Bueren & Baars, 2003.

Natural Entity Beyond Europe

As of now, there is no federal or state legislation in Australia that explicitly uses the term *natural entity* in the way Spain does to confer legal personhood on ecosystems. However, there is growing interest in rights-of-nature frameworks, particularly in environmental legal scholarship and Indigenous governance.

Australia's common law system remains strongly focused on human impacts and interests in environmental regulation. That said, also in Australia, Indigenous legal traditions, such as those of the Yorta Yorta (Victoria and New South Wales), Yolngu (Northern territory), and Martuwarra Fitzroy River peoples (Kimberley, Western Australia), have long conceptualized rivers and species as law-bearing beings. These traditions inherently treat natural systems as entities with moral and spiritual agency, thus closely aligning with the term *natural entity*. An illustrative example is the Martuwarra (Fitzroy River) Declaration (2016)[42], in which the 'Traditional Owners' recognized the river as a living ancestral being with its own law and governance structures. Although not legally binding under Australian law, such declarations do form the basis of Indigenous-led environmental governance and are increasingly cited in political and academic discussions about the future of ecological personhood in Australia (Reed, Brunet, Longboat & Natcher, 2020; Davis, 2025).

Several local governments in the United States have adopted rights-of-nature decrees, similar to Spain, referring to ecosystems as natural entities. For example, the Lake Erie Bill of Rights[43], passed in Toledo, Ohio in 2019, stated that "Lake Erie, as a natural entity, possesses the right to exist, flourish, and naturally evolve." Although the ordinance was later struck down in court, its language continues to influence community-based environmental activism and legal reform efforts. Other examples include legal efforts to recognize the rights of rivers in the States of Florida[44] and Pennsylvania[45], often spearheaded by local governments or tribal nations. These initiatives reflect growing public support for extending legal standing to ecosystems under the label of 'natural entity'. While Canada has not yet codified the term natural entity in its statutes, proposals in provinces such as Quebec and British Columbia have considered granting legal personhood to rivers and other ecosystems, for instance, the Magpie river (or Muteshekau Shipu) in Quebec[46]. Indigenous legal traditions often treat rivers and mountains as law-bearing entities. These traditions align closely with the notion of natural entity, and open pathways for integrating natural entity terminology into future Canadian law. Notably, some First Nations and Indigenous communities in Canada have unilaterally declared rights for rivers and territories under their traditional governance systems.

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[42] https://ecojurisprudence.org/initiatives/fitzroy-river-declaration-recognizing-martuwarra-fitzroy-river-as-a-living-ancestral-being/
[43] https://www.utoledo.edu/law/academics/ligl/pdf/2019/Lake-Erie-Bill-of-Rights-GLWC-2019.pdf
[44] https://geog.ufl.edu/2020/04/22/rights-of-nature/
[45] Mentioned in the 'Frack-ban order', part of the Pittsburgh City Ordinance 37-2010. In this ordinance, the 'rights-of-nature' clause declares that "natural communities and ecosystems, including wetlands, streams, rivers, aquifers and other water systems, possess inalienable and fundamental rights to exist and flourish. Available via https://pittsburgh.legistar.com/ViewReport.ashx?GID=115&GUID=LATEST&ID=13487&M=R&N=TextL5&Title=Legislation+Text
[46] https://allard.ubc.ca/about-us/blog/2021/rights-nature-and-indigenous-peoples-navigating-new-course