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# Is defining life pointless?

## Operational definitions at the frontiers of Biology

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**Abstract:**

Despite numerous and increasing attempts to define what life is, there is no consensus on necessary and sufficient conditions for life. Accordingly, some scholars have questioned the value of definitions of life and encouraged scientists and philosophers alike to discard the project. As an alternative to this pessimistic conclusion, we argue that critically rethinking the nature and uses of definitions can provide new insights into the epistemic roles of definitions of life for different research practices. This paper examines the possible contributions of definitions of life in scientific domains where such definitions are used most (e.g., Synthetic Biology, Origins of Life, Alife, and Astrobiology). Rather than as classificatory tools for demarcation of natural kinds, we highlight the pragmatic utility of what we call *operational definitions* that serve as theoretical and epistemic tools in scientific practice. In particular, we examine contexts where definitions integrate criteria for life into theoretical models that involve or enable observable operations. We show how these definitions of life play important roles in influencing research agendas and evaluating results, and we argue that to discard the project of defining life is neither sufficiently motivated, nor possible without dismissing important theoretical and practical research.

**Keywords**

Definitions of Life; Integration; Origins of Life, Artificial Life; Synthetic Biology; Astrobiology; Philosophy of Science in Practice.

## 1. Introduction

The fundamental question of *what life is* was for many years set aside by the success of experimental studies of molecular structures in Biology, but the issue has recently gained increasing popularity and attention (Gilbert & Sarkar, 2000; Woese, 2004; Cornish-Bowden, 2006; Di Frisco, 2014; Nicholson, 2014; Moreno & Mossio, 2015). Accordingly, the more specific issue of *defining* life has also raised renewed interest in Biology and Philosophy. Whereas many 20th century biologists considered the problem of defining life a purely theoretical or even metaphysical question, often characterised by a compromising vitalist flavour (Mayr, 1982), several new fields now take this to be a fundamental question. Synthetic Biology, Origins of Life, Astrobiology, and computational Artificial Life are examples of fields in which definitions of life have become an established research topic connected to the characterisation, detection, demarcation, and synthesis of life (Luisi, 2006; Ruiz-Mirazo et al., 2010). The renewed attention to definitions is, however, also followed by renewed criticisms. It is our aim in this paper to examine the arguments of these criticisms in comparison to the roles that definitions of life play in scientific research practices.

The background for the criticism is the lack of consensus among scholars who have proposed definitions of life. Despite various and long-lasting efforts to define life, there is no agreement on the central features that unite living systems and separate these from inanimate matter. More generally, there is no shared position on the importance of definitions of life in the practice of science (Szostak et al., 2001; Ruiz-Mirazo et al., 2010; Tirard et al., 2010; Trifonov, 2011; 2012; Szostak, 2012).

A few criticisms from philosophers stand out as radically undermining the project of defining life, and as possibly leading to the abandonment of this enterprise as pointless or even detrimental to the sciences involved. Against the background of diversity of definitions, Edouard Machery recently argued that: “the project of defining life is either impossible or pointless” (Machery, 2012, 145). Insofar as this enterprise is spread over many disciplines with diverging interests, preferences, goals and research traditions, and insofar as it is also possible that life might not even form a single natural kind, he argues that a consensus cannot be reached. In parallel, Carol Cleland proposes four main arguments against definitions of life (Cleland & Chyba, 2002; 2007; Cleland, 2012). The first and more fundamental, she sustains, is that definitions are limited conceptual tools that cannot express necessary and sufficient conditions for a natural kind. Second, she criticises the use of definitions as theoretical identity statements by pointing to their incapability to encapsulate scientific theories and the distinctive properties of the objects they describe. Third, she argues that definitions are likely to make scientists blind to other possibilities (e.g., alternative life forms) and therefore are likely to mislead us. Fourth, she argues that since we only know one example of life (the one found on Earth), there is no well-developed general theory of life available upon which to rely in order to distinguish necessary from contingent properties of life[1].

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[1] We address the first criticism in Section 2.1, and the others in section 4.2.

In the face of such criticisms ─ and, indeed, precisely by taking them into account ─ this paper aims at clarifying the importance of definitions of life in scientific practice and in the theoretical advancement of the disciplines involved. The real point of contention, we argue, is what is taken to count as a definition, and for what purposes definitions are offered (or taken to be offered). Rather than taking for granted that the role of definitions in science is equal to the demarcating natural kinds in analytical philosophy, we approach the issue of definitions from a pragmatic standpoint. We focus on the possible contributions of definition to the research carried out in those domains in which are used most (Synthetic Biology, Origins of Life, Alife, and Astrobiology).

By analysing the practical utility of definitions of life in scientific practice, we propose that definitions of life in these domains should be considered in a non-standard and weaker sense, as *operational definitions*. This choice of terminology is inspired by the use of the same term in the scientific literatures of Origins of Life and Synthetic Biology (e.g., Fleischaker, 1990; Luisi 1998). We use the term 'operational' in a wide sense, referring both to (1) the possibility to define something by means of operations (e.g., defining an entity by measuring or building it following a specific procedure), and (2) the idea that the contents of the definition (e.g., the conditions for life) can be operationalised for empirical research, that is, can be built, manipulated and tested in the laboratory. We are aware that operational definitions have slightly different uses in other philosophical debates and domains of science, related to the first meaning.[2] Yet, we choose to adhere to its use in the scientific literature and refer by 'operational definitions' to the second meaning as well (for a more detailed discussion, see Sections 3 and 4).

Importantly, operational definitions imply that their content can be object of experimental or formal scientific analysis. Properties that cannot be objects of scientific research because of practical reasons, for example due to limits in the technological or formal tools available at a certain moment, or metaphysical dispositions like *entelechies*, cannot be part of an operational definition in this sense. Operational definitions of life are connected to specific theoretical models which integrate sets of contextually relevant criteria for life that involve or enable observable or experimental operations (in the laboratory, on another planet, or in a software program). This means abandoning several features usually associated with definitions of life in the philosophical debate, such as strict ontological claims associated with definitive or stipulative demarcations of necessary and sufficient conditions for life, that are fixed once and for all and demarcate life as a natural kind.

We first examine the background of the debate and the main challenges to definitions of life, brought forth by Machery and Cleland (Section 2). Even though these criticisms call for a critical rethinking of the nature and role of definitions, we argue that the pessimistic conclusions do not follow once strong ontological claims on the relation between definitions and natural kinds are abandoned. Section 3 discusses the utility of definitions of life in fields that explore the frontiers of Biology. Specifically, we analyse the role played in the line of investigation pursued by Pier Luigi Luisi's research group within Origins of Life and Synthetic Biology. In Section 4, we propose that an operational account better captures the use of definitions of life in these fields, and we respond to possible objections.

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[2] The way we use the term here should also not be confused with the broader notion of *operationalism,* i.e., the view of some logical positivists that the meaning of a term bears solely on the methods for its empirical measurement.

Section 5 concludes with some general remarks on the distinction between operational and strong definitions.

## 2. Philosophical challenges to the attempts to define life

The difficulties of catching the distinctive and universal character of living systems and of reaching a consensus on a single definition ─ due to the great diversity of living phenomena, which ‘resists the confines of any compact definition’ (Szostak et al., 2001: 387) ─ do not seem to have discouraged the enterprise to define life. Rather, attempts from distinct disciplines and research traditions have multiplied. Popa’s extensive work (2004) lists almost 100 different definitions of life, not counting more recent contributions, among these are for instance several organizational accounts (Ruiz-Mirazo et al., 2010; Bich & Damiano, 2012) and cybernetic approaches (Tsolokov, 2010), just to cite few of them.

In the face of this variety, several attempts to systematize the debate and advance unifying definitions have been made (Kompanichenko, 2008; Trifonov, 2011). In this context of intense discussion and implementation of different strategies (Szostak, 2012; Trifonov, 2012), several journals have recently dedicated special issues to definitions of life: *Origins of Life and Evolution of Biospheres* (2010, 40-2), *Synthese* (2012, 185-1), and *SEBBM* (2013, 175). In addition, one of the four sections of the comprehensive book edited by Bedau and Cleland on the *Nature of Life* (2010, Sec IV) is specifically dedicated to contributions on this topic. Papers in these publications defend, challenge or profoundly criticise the general quest for definitions. Moreover, criticisms have also been made of the use of definitions in specific fields, namely Astrobiology (Cleland & Chyba, 2007; Cleland, 2012) and Origins of Life (Szostak, 2012). The following subsection describes in further detail the philosophical criticisms by Machery and Cleland.

### *2.1 Definitions of life and natural kinds*

In light of the proliferation of definitions of life, Machery (2012) argues that if defining life is a scientific project like defining gene, virus, cell etc., then the project is pointless because different scientific disciplines, or even research groups within the same discipline, do not reach a consensus[3]. A central assumption in Machery’s argument is that the project of defining life is one of finding one unified conceptual definition that covers all and only the relevant life forms, and on which all scientists would agree. The idea is that the aim of defining scientific concepts is to arrive at consensus on necessary and sufficient conditions for specific terms or classes – an aim that seems impossible to reach.

One problem with definitions of life results from the diversity of preferences for definitions held by individual scientists with different disciplinary and theoretical backgrounds as well as different research agendas. For instance, scientists interested in uncovering the origins of life are interested in defining minimal biochemical life forms that may not be compatible with strong accounts of Artificial Life, that argue that life is independent of materiality. Similarly, astrobiologists interested in

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[3] In the same paper Machery (2012) also proposes an argument against definitions of life in folk psychology. We will not address it here, as we are interested in their use in science.

understanding what components and organisations could support life on other planets, and how to detect it, may have other restrictions for a satisfactory definition of life. Moreover, the prospects of finding one unified definition that covers all and only the relevant life forms are challenged by the fact that it is "unclear whether living beings form a single natural kind since nature rarely yields a unique way of classifying the world" (Machery 2012: 159). In other words, we may be trying to identify the boundaries of a group of entities that upon closer inspection do not form a single natural kind.

Cleland's argument, grounded in metaphysics, is even more radical. She claims that definitions in general are incapable of answering questions about natural kinds and are therefore useless for scientific investigation. Her criticism leans on Putnam's (1975) famous Twin Earth Example, which she uses to argue that the extension of a term is not fully determined by the concepts in the mind (Cleland, 2012; see also Kripke, 1972). The Twin Earth Example[4] suggests that insofar as definitions are dependent on concepts formed by subjective and fallible features of human thoughts and language, they cannot provide necessary and sufficient conditions for demarcating natural categories or entities. Yet, according to Cleland, this is precisely what is usually required of them. Hence, if scientists and philosophers alike are interested in demarcating and identifying life as a natural kind ─ or in establishing for example whether an entity such as a virus is alive or not ─ *in a strong ontological sense*, then appealing to a definition is a wrong choice. Moreover, in Cleland's view the use of definitions carries also practical issues and risks, insofar as it may contribute to entrenching a misconception or just restate what scientists already know (see Section 4.2).

Machery's and Cleland's criticisms seem to leave no other choice but to abandon the enterprise of defining life, since definitions cannot provide univocal necessary and sufficient conditions for life. Importantly, however, their criticisms primarily target the capability of definitions *to mark out natural kinds*. We stress that there are other ways to understand the role of definitions by focusing on their uses in scientific practice.

### *2.2 An alternative pathway: disengaging from strong requirements on natural kinds*

The debate on natural kinds has itself generated a variety of incompatible theories and raised criticisms. In particular, if definitions are understood as attempts to demarcate static ontological categories with very strict and rigid boundaries, they are vulnerable targets of Machery's and Cleland's criticisms. But our claim is that this characterisation of definitions is inadequate to account for the role of definitions of life in scientific practice. An alternative approach, advanced by Goodman (1983), Brigandt (2011), and others, invokes a pragmatic or instrumental use of natural kinds as

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[4] The famous example considers two planets which are exactly the same except that the substance they call water, and which exhibits the same sensible properties, has a different composition on each planet. Before such chemical composition is discovered, two identical individuals with identical mental states, living on the two planets, would both call the substance 'water'. They have the same concept of what water is and would think water is the same on both planets. But once scientists discover the different chemical composition of the two substances on the respective planets, they show that using the same term is wrong: "It follows that the extension of the term 'water' is not fully determined by concepts in the mind" (Cleland 2012: 134). Putnam's example has been strongly criticised, as admitted by Cleland herself. Yet, she claims, it exposes an uncertainty in the relationship between concepts and natural kinds that is sufficient to undermine definitional approaches.

research tools, rather than attempts to establish a unique and best way to classify things in nature, by stressing their practical role over their ontological value. According to this account, different ways of classifying things can be chosen according to specific scientific goals and evaluated in terms of their usefulness to scientific practice.[5] We share with this approach the view that definitions of life inform inferences about central properties of systems, and thus serve important practical and theoretical roles. The philosophical discussion of whether life is a natural kind or not, we suggest, is not the only, or even most important, aspect of defining life. Rather, we are interested in the concept of life as a practical and theoretical target in research that aims to investigate the origin of life, design life, or address the "salient puzzles about life" (Bedau, 1998: 125; see also Wolfe, 2014).

Definitions of life play a role akin to definitions of other scientific concepts such as ‘gene’, ‘disease’ or ‘organisms’ which play important roles in scientific and philosophical discourses despite a lack of consensus or optimism that these correspond to distinct or univocally definable natural kinds (Dupré 1993; Moss 2001; Scully 2004; Waters, 2006; see also Section 4.2). Like these concepts, the notion of ‘life’ can be seen as a conceptual tool for different experimental systems and research programs. It can play an important role in the theoretical activity of comparing, through references to definitions, different types of systems that are considered as limit cases with epistemic implications (Wolfe, 2014). Examples are viruses (Forterre, 2010) and *transient* systems at the frontier between Chemistry and Biology (Etxeberria and Ruiz-Mirazo, 2009). Such conceptual boundaries can be complex and dynamic, and their choice has great theoretical and practical implications in the elaboration of scientific programs (Forterre, 2010). From this perspective, unification is not the only – or even most relevant – criterion for success. Rather, definitions of life – despite their diversity – reflect ongoing attempts to understand the complexity of the central properties of life from different perspectives (see also Malaterre, 2010).

In the following, we shall examine the role definitions of life in terms of their contributions to the theoretical and experimental understanding of life that they support or facilitate.

## 3. Defining and redefining life in practice

Definitions of life play prominent roles in recent interdisciplinary research areas at the frontiers of Biology, such as Synthetic Biology, Origins of Life, Astrobiology and computational Artificial Life. These fields draw on Engineering, Biochemistry, Physics, Computer Science, etc. in their attempt to design and understand systems at the *edge of life*. This section illuminates the practical role of definitions of life in science by analysing the work of the biochemist and synthetic biologist Pier Luigi Luisi ─ and of his team at ETH-Z, Zurich and University of Roma Tre ─ in the investigation of the *transitions to life* (Luisi, 1993)[6].

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[5] According to Brigandt, "[f]or any kind, the philosophically relevant question is an epistemic issue: how scientifically important is the grouping of an object into a kind, i.e., what generalizations and explanations can the kind figure in, and how important are they?" (Brigandt, 2011). See Dieguez (2013) for a position that combines ontological and practical claims. See Amilburu (2015) for a recent detailed classification and discussion of different approaches to natural kinds.

[6] The analysis is based on published papers as well as personal interactions between one of the authors and Luisi and his team for more than a decade.

### *3.1 The case of Luisi's team*

Luisi has been a leading scientist at the crossroads of some of the fields mentioned above, and since the early 90’s he has made explicit use of definitions of life. In his work, definitions play a fundamental instrumental role in informing experimental research and in the generation of novel knowledge:

> Why should one have a definition of life? (…) A definition of life illustrates an experimental program: once you have the intellectual clarification of the definition in front of you – there you have a challenge to implement it in the laboratory (Luisi, 1998: 621).

According to Luisi’s pragmatic view, definitions are directly related to the aims and goals of the scientific user, and their ‘success’ is not evaluated in terms of unification or consensus. Rather, their utility depends on practical purposes, and "one definition may be more meaningful than another, depending on what you want to do with it" (Luisi, 1998: 617).

Luisi is known, among other things, for his research on protocells, and more specifically on the synthesis of fatty acid and lipid compartments: from micelles to vesicles[7]. This research line, started in the early 90’s after meeting Francisco Varela, was initially based on the following definition of living system:

> "a system which is spatially defined by a semipermeable compartment of its own making and which is self-sustaining by transforming external energy/nutrients by its own process of component production" (Luisi, 1998: 619).

It is a reformulation of the definition of living system as an autopoietic organisation given by Maturana and Varela ([1973] 1980)[8], and draws on a theoretical framework, focused on the idea of organisms as autonomous systems (Moreno & Mossio, 2015). A characteristic of this framework is that it attempts to put together into a coherent conceptual formulation what are considered the crucial properties common to all living systems. Specifically, it integrates metabolism (self-production) and compartmentation (self-distinction from the medium and control over concentrations and exchanges) into an organised system capable of achieving self-maintenance as a whole.

The use of a definition of life as an autopoietic system ─ formulated in a way that explicitly stresses the importance of compartments ─ had an important theoretical impact on the study of the origins of life. The synthesis and study of different kinds of compartments has a long history (Hanczyc, 2009).

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[7] A micelle is a spherical aggregate of lipid molecules characterised by a hydrophilic polar head directed towards the solvent and a hydrophobic tail directed towards the interior. A lipid vesicle is a structure characterised by a fluid core enclosed by a lipid bilayer. See Stano and Luisi (2016), for a recent historical review of the main research lines developed by Luisi’s research teams in Zurich and Rome.

[8] “[The autopoietic organisation] (...) is a network of production processes (transformation and destruction) of components which produces the components which: (1) Through their interactions and transformations, permanently regenerate and realize the network of processes (relations) which produces the components; and (2) constitute a concrete unity in space, within which they (the components) exist by specifying the topological domain of its realization in that network.” (Maturana and Varela [1973] 1980: 79).

But the theory of autopoiesis is among the first contributions that explicitly moved the focus of research towards the specific question of the construction of the membrane from within the system, in the more encompassing context of the self-production of the organism. Due to this very feature, the notion of autopoiesis has played a central role in integrating approaches focused on metabolism and compartments in experimental and computational research[9].

This theoretical approach has informed Luisi's experimental research by guiding it towards types of compartments, such as vesicles, capable, in principle, not only of harbouring metabolism, but also of being generated and maintained by it (Luisi, 1993). Several experiments inspired by the autopoietic definition have been performed over the decades. One example is Zepik et al.'s (2001) chemical model of minimal autopoietic unity (Figure 1), developed to study the relationship between compartments and self-maintenance in the prebiotic world (see also Luisi, 2015). The experiments carried out in this work are based on self-producing oleate vesicles, that constitute an example of compartmentalised protocells[10]. The boundary of the vesicle is maintained by the continuous replacement of oleic acid components (*S* in the figure) on the surface through the hydrolysis of a precursor *A*. The originality of Luisi's chemical model derives from the fact that the anabolic reaction of vesicle production (representing growth) is combined with a competitive oxidation reaction, which destroys the membrane oleate components (representing catabolism or decay). In this reaction, *S* is transformed into the decay product *P*. By balancing the relative concentrations of the reagents, and thus the velocities of the two reactions, Zepik et al. (2001) obtained a chemical model that could account for different and biologically interesting kinetic modes such as homeostasis (when the velocities are equal), growth (when production is faster than decay, eventually leading to division and self-reproduction), and death (when decay is faster than production). The chemical model, thus, allows for the exploration of different possible dynamic regimes by modulation of these reactions, providing a proof of principle for experimental investigation of possible self-maintaining precursors of current living systems[11].

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[9] Varela et al. (1974) proposed the definition of an autopoietic system together with a computational model of the generation and maintenance of a compartment. Thus, early research on Artificial Life was directly related to a specific definition. The relationship between the metabolism and the compartment had been stressed in the same years by Gánti (1979) through his model of the chemoton, thought of as a possible realisation of a definition of a minimal living system. Some of the first (but ultimately unsuccessful) experimental attempts to synthesise an autopoietic system were performed by Gloria Guiloff, a graduate student in Maturana's laboratory at the Universidad de Chile (see Guiloff, 1981).

[10] Oleate vesiscles are spherical bilayer structures that host an aqueous core, and are composed of simple long chain fatty acids (such as oleic acid) that are ionised to form hydrogen bonds.

[11] Today, more sophisticated forms of such experiments are common both in wetware and software domains, but at the time they were unusual (see Luisi, 2015). For a comprehensive review of this approach in current systems chemistry see Ruiz-Mirazo et al. (2014). For recent examples of wetware and software applications see Murillo-Sanchez et al. (2016) and Agmon et al. (2016), respectively.

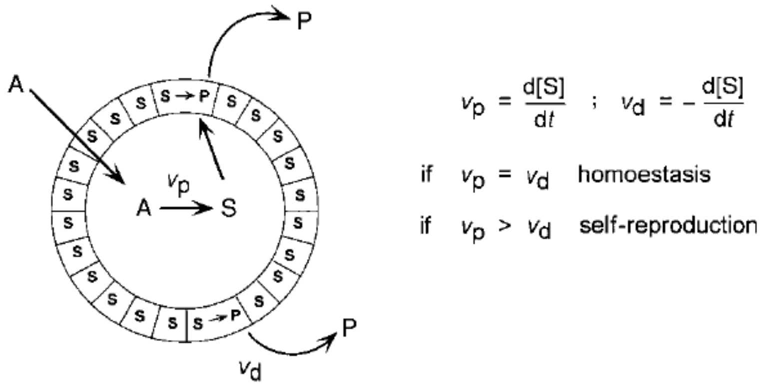

Figure 1. The chemical model of the “minimal autopoietic unit”: an experimental system directly inspired by Luisi’s definition of life. The system is characterised by two competitive reactions of anabolism and catabolism. S represent the oleic acid components of the compartment, A the precursors, and P the decay products. Figure from (Zepik et al. 2001), Copyright © 2001 by John Wiley & Sons, Inc. Preprinted by permission of John Wiley & Sons, Inc.

Additional insights into the role of definitions of life can be derived from Luisi’s work. Definitions not only specify individual research trajectories, but also facilitate collaborations and intersections between different perspectives. Moreover, they can be combined to generate novel research approaches. For example, the common interest in understanding minimal life, by combining internal processes and compartments, has brought together scientists with diverging views and definitions of life such as Szostak, Bartel, and Luisi. The former two support the evolutionary view, while the latter the autopoietic view based on autonomy, but these came together in the following proposal:

> How simple can a cell be and still be considered as living? The answer depends on what we consider to be the essential properties of life. Defining life is notoriously difficult; its very diversity resists the confines of any compact definition. An operational approach focuses on identifying simple cellular systems that are both autonomously replicating and subject to Darwinian evolution (Szostak et al, 2001: 387).

The cited paper became the manifest of a full-fledged research program. Its long-term objective was to develop systems of vesicles capable of encapsulating biologically relevant molecules and hosting compartmentalised biochemical processes. Furthermore, the vesicles should be capable of evolutionary change, with selective pressures arising from functionally advantageous genetic changes and vesicle replication[12]. The relationship between template-directed synthesis of genetic polymers and vesicle replication has been explored by Szostak’s group as a step towards the realisation of the

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[12] See Oberholzer et al. (1995) for a preliminary realisation of this idea in oleate vesicles by Luisi’s team at ETH-Zurich. This work already shows a flexible attitude towards combining different definitions: "by combining the RNA replication with the principles of autopoiesis, we obtained a bridge between the two more accepted views on the theory of minimal life, the one based on the “RNA-world” and the other based on the cellular autopoietic view” (See Oberholzer et al., 1995: 255-256).

coupling between template replication and compartment division (Mansy et al, 2008; Adamala & Szostak, 2013)[13].

In parallel, the attempt to encapsulate molecules in protocells by Luisi's group has revealed unexpected and interesting results that are still not fully explained (Luisi et al, 2010; Souza et al, 2014). One of the poorly understood aspects of the origin of life is how self-maintaining protocells can arise from separate constituents. For instance, a central question is how molecules, such as catalytic RNA (ribozymes) or small peptides, could become entrapped or permeate into a lipid compartment and start interacting. Experiments on vesicle formation can illuminate some possible answers to how this could have happened. Luisi's group expected that when vesicles form in diluted solutions in the presence of ferritin or ribozymes they exhibit similar (low) concentrations of entrapped molecules. However, they observed that most of the vesicles are empty. Yet, few of them (about 1%) entrap a very high number of solutes and achieve molecular crowding, possibly favouring the beginning of a primitive compartmentalised metabolism (Figure 2).

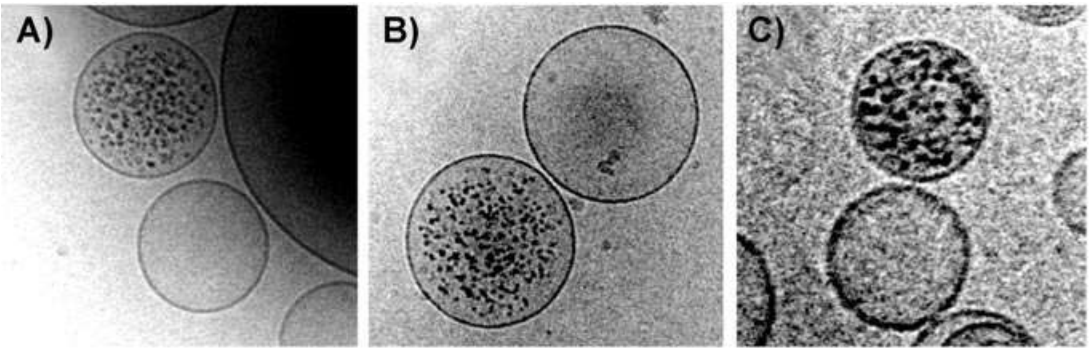

**Figure 2**. Entrapment of the protein ferritin inside lipid vesicles. Some vesicles are empty while others exhibit a high concentration of ferritin molecules. Figure from (Luisi et al. 2010), Copyright © 2010 by John Wiley & Sons, Inc. Preprinted by permission of John Wiley & Sons, Inc.

These experiments represent fundamental steps in a research line that is characteristic of a wider theoretical approach to the study life and its origins, based on the notion of systemic integration (Bich, 2010). This approach currently numbers several attempts to integrate membrane and metabolism, template and membrane, and metabolism and template in synthetic prebiotic systems (reviewed in Ruiz-Mirazo et al., 2014).

### *3.1 Evolving definitions*

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[13] Szostak has recently criticised the effort of defining life on the grounds that the origins of life concern transitions, but definitions of life do not tell us how these transitions took place (Szostak, 2012; see also Trifonov, 2012). Yet, although definitions do not tell what happened, they can guide the scientist in selecting which features to examine: they are not answers, but tools. Szostak himself defines life in terms of Darwinian evolution, considered as the "unifying characteristic of all Biology" (Szostak, 2012: 599). Accordingly, he focuses primarily on realising in the laboratory those transitions that give rise to conditions for evolution, such as the combination of template replication and protocell division (see Mansy et al., 2008; Adamala & Szostak, 2013).

An important aspect of operational definitions is that they are revisable in response to challenges, new insights, or criticisms that point to weak spots of the theory on which they are based. Definitions, as employed by Luisi and colleagues, have changed over time following the results of theoretical reflection and experimental research.

For example, the biological pertinence of the model of a chemical autopoietic system (Fig. 1) developed by Luisi and collaborators on the basis of Luisi's definition of a living system (Zepik et al, 2001), was theoretically and philosophically questioned by Luisi himself and Michel Bitbol (Bitbol and Luisi, 2004). The chemical model appears too limited if compared to a minimal organism such as a bacterium. Only few (simplified) reactions take place in the chemical system, whereas a full-fledged living system exhibiting metabolism is capable of interacting with the environment by reacting conservatively to external perturbations. This aspect is not explicitly addressed by the definition (Luisi, 1998) and by the experimental model inspired by it (Zepik et al, 2001), even though it is part of the more general theory of autopoiesis. Hence, this reflection led to the reformulation of the definition of life based on autopoiesis, with the incorporation of another aspect of the theory which previously was not included: cognition, in its minimal meaning of the capacity to interact with the environment and respond to changes in it (Damiano & Luisi, 2010; Bich & Damiano, 2012). According to the 'redefined' version, "a living system is a system capable of self-production and self-maintenance through a regenerative network of processes which takes place within a boundary of its own making *and regenerates itself through cognitive or adaptive interactions with the medium*." (Damiano & Luisi, 2010: 149, italics ours). This example shows that definitions can change within the framework provided by a specific theory.

Another interesting aspect of operational definitions is that attempts to respond to challenges for the theoretical framework on which they are based can lead to modifications or to novel experimental results. Research based on the autopoietic definition of life traditionally focused on individual cells (represented as individual vesicles). The focus is justified through the interest in the simplest unit of life. Yet, the relatively recent recognition in the microbiology community that unicellular organisms live in colonies (see for example Costerton et al. 1995; Dupré and O'Malley, 2009) has given support to the idea that life might have arisen from *cooperative mechanisms* between prebiotic systems (Carrara et al. 2012). Moreover, experiments with single vesicles (especially liposomes[14]) give limited results due to their low permeability, and they incur into difficulties in realising an exchange of materials between individual vesicles. In response to such challenges, Luisi's group investigated the properties of *colonies* of giant vesicles (Carrara el al., 2012, Figure 3), an approach previously unexplored in the literature. Using oleate-based vesicles allowed the researchers to explore functional features of a negatively charged membrane. They observed that the vesicles in contact with polycations form physically stable colonies which attach to the solid substrate (like biofilms) and could attract positively charged compounds at the surface.

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[14] Liposomes are vesicles composed of phospholipids, the lipids that compose current cell membranes. They are more stable but less permeable than oleate vesicles.

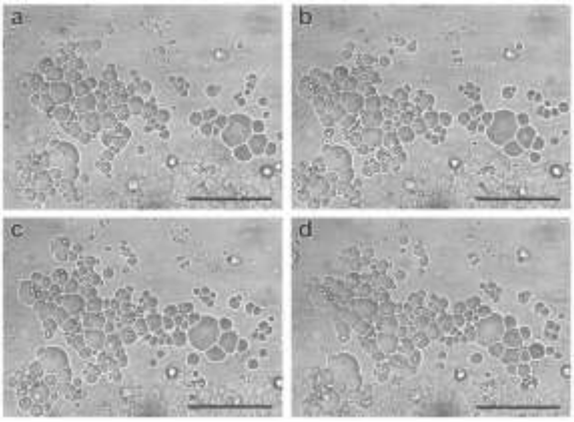

**Figure 3.** Reciprocal attraction of colonies of giant vesicles. See text for details. From (Carrara et al. 2012), Copyright © 2012 by John Wiley & Sons, Inc. Preprinted by permission of John Wiley & Sons, Inc.

Importantly, the study led to the unexpected discovery that vesicle colonies, compared to individual vesicles, have features that better mimic some biological processes. These include a demonstrated increased permeability and a capability to incorporate solutes. Moreover, using fluorescent t-RNA the group showed how also larger compounds can attach to the colony surface and more slowly penetrate the membrane without breaking the vesicles' macrostructure. The latter operationalisation corresponds to a possible scenario of the origin of metabolism where externally formed polymers are captured by primitive compartments. Moreover, they observed colony accretion ─ which is also observed in bacterial biofilms ─ vesicle fusion, and exchange of material between vesicles (Carrara et al., 2012). In this case, the debate on the limitations of current operational criteria for studying the origins of life improved both the definition and the experimental models of primitive cell communities. This example also shows that definitions can provide a context for the discussion and revision of fundamental theoretical assumptions.

### *3.2 General roles and applications in different fields at the frontiers of Biology*

The case of Luisi's group shows that definitions are used and can be useful as operational tools to guide research in Origins of Life and Synthetic Biology. More generally, definitions can play a theoretical, epistemological, practical, and even ethical role[15] in several disciplines at the frontiers of biology. Their content can range from life considered as a collective phenomenon ─ the living world or the biosphere in general[16] ─ or as the distinctive character of any individual living system.

Definitions play an important role in fields where research is guided, at least in part, by explicit or implicit assumptions on the difference between living and non-living systems, and where the goal is theoretical advancement through analysis, challenging, testing and subsequent improving of models

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[15] The role of definitions of life related to ethics has ramifications that extend to environmental ethics and medicine (Machery, 2012). The role of definitions of life in ethics is beyond the scope of this paper, which is focused on the role played by definitions in the frontier disciplines aforementioned.

[16] This is the case, for example, of those approaches which include Darwinian evolution as a crucial property to define life.

of living systems. They are often used in the construction of theoretical models[17] aimed at explicitly characterising or simulating some crucial features of basic biological systems as well as of *unfamiliar forms of life* (Rasmussen et al, 2008), and *transient systems* (Etxeberria & Ruiz-Mirazo, 2009) such as prebiotic systems and protocells.

The epistemological role of definitions of life is related to the specific goals and distinctive features of the different domains of investigation in the research fields mentioned above. Definitions may provide insights and guidance in the design of experimental[18] or computational research programs[19], in the synthesis of proto-biological systems[20], or in the realisation of biological relevant properties[21]. Furthermore, they are sometimes used in order to identify or detect living systems[22], or to distinguish them from non-living ones[23].

Different fields can have different contents and criteria of "practical operationability" (Luisi, 1998), i.e., the actual uses for definitions as tools to guide and inspire experimental research and theoretical debate. Yet, discussions and hybridisations often cut across disciplinary boundaries, as do many of the scientists involved. Origins of life research often relies on definitions in orienting the investigation of the fundamental steps towards, or requirements for, life and applies them in the design and construction of plausible prebiotic systems and properties. In research on protocells[24] definitions are used to provide criteria of pertinence and relevance in the design of biologically significant systems (e.g., Rasmussen et al., 2008; Stano & Mavelli, 2015). For example, they are used to guide decisions regarding which basic functions should be included in the protocells in order to make them plausible models of possible precursors of life. Similarly, in some branches of Synthetic Biology definitions play a role in the choice of relevant or interesting biological properties to implement in synthetic systems (Ruiz-Mirazo & Moreno, 2013). Moreover, as acknowledged in the literature in Synthetic Biology, the evaluation of results in the design of synthetic cells is particularly problematic in the absence of definitions (see for example, Cronin, et al., 2006; Forlin et al, 2012)[25].

In Artificial Life definitions are used, for example, in the selection of the relevant properties of life that should be simulated. Moreover, this field has been characterised by intense theoretical debates on the possible domains of realisation of life. For instance, it has been debated whether life requires metabolism, and therefore a chemical and molecular domain (Rosen, 1991; Boden, 1999; Moreno and Etxeberria, 2005), or whether life could be realised regardless of the material substrate, even in

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[17] Or are identified with theoretical models of minimal living systems (see Letelier et al., 2011)

[18] As argued in Ruiz-Mirazo & Moreno (2013). Examples of this use of definitions are Guiloff (1981), Fleischaker (1990), Luisi (1993), Murillo-Sanchez et al. (2016).

[19] Discussed in Boden (1999). Examples are Ruiz-Mirazo & Mavelli (2008), Piedrafita et al. (2010), van Segbroeck et al. (2009), Zachar et al. (2011), Shirt-Ediss et al. (2014), Agmon et al. (2016).

[20] Discussed in Ruiz-Mirazo & Moreno (2004), Bich & Damiano (2007). Examples are Szostak et al. (2001), Zepick et al. (2001), Stano & Mavelli (2015).

[21] For example Rasmussen et al. (2008), Mansy et al. (2008), Luisi et al. (2006).

[22] Discussed in Raulin (2010), Cyzweska (201). See Cleland (2012) and Bains (2014) for alternative views.

[23] See for example Forterre (2010).

[24] Protocells are coherent unities (spherical collections of lipids) proposed as the *infrastructures* for the origins of life. See Shirt-Ediss (2016) for a thorough analysis of the protocells approach to study the origins of life.

[25] An alternative proposal advanced by Cronin et al. (2006) has been to design and implement Turing tests for *lifeness*, to have real cells evaluate artificial ones. Yet, the value of the test is only limited to *life-like interactions*.

software simulations (Langton, 1989; Ray, 1992; Grand et al, 1996)[26]. Definitions play a primary role in Astrobiology as well[27], where the difference in criteria of *practical operationability*, compared to other disciplines, is particularly striking. In this domain, definitions function as sources of criteria for the *detection* of life, rather than for the *design* and *construction* of it. Systems Biology[28] and Prebiotic Systems Chemistry[29] are other examples of fields involved in developing an integrated account of biological systems where definitions of life have become more prominent.

In summary, in the development of fields working on problems regarding the characterisation, detection, design and synthesis of life, definitions of life play important roles as boundary concepts for specifying research approaches and specific operations. These examples provide empirical resistance to the assumptions that most biologists take life to be a natural kind and that the purpose of definitions of life in science is to demarcate it (e.g., Cleland, 2012: 127). In the cases we have examined, even the heuristic use of definitions to establish whether something is alive or not, is actually marginal, compared to their more general role as tools in actively guiding research. In the following section we will attempt to systematise this operational view of definitions as opposed to an ontological one, and answer possible objections.

## 4. Operational definitions and their virtues

We have shown that, in several fields, definitions of life are used in a way that is not necessarily related to carving out natural categories in a strong ontological sense, and their value does not depend on consensus, but rather on their impact on research. Scientists pragmatically define life consistently with the current and continuously changing biological theory as well as according to their own specific theoretical and experimental perspective and goals. The examples taken from the work of Luisi and colleagues show this instrumental use for definitions: they are directly involved in scientific practice, and play an active operational role in guiding theoretical and experimental research, and in generating novel knowledge.

In the examined examples, definitions are exploratory tools that can change over time and across research programs. This use does not imply a requirement of a unique, complete and definitive set of necessary and sufficient conditions for all life (and only life) as we know it, or the commitment to strong ontological claims. Instead, such tools point out some *possible necessary conditions* for life which are considered plausible and interesting *in the context of* current theories and available experimental techniques[30]. In doing so they aim at identifying a set of conditions that are *satisficing*

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[26] For a discussion of the role of definitions of life in Artificial Life, see for example Umerez (1995).

[27] Consider for instance the NASA effort to formulate a definition to help decide which experiment to realise to detect life on Mars: "Life is a self-sustained chemical system capable of undergoing Darwinian evolution" (discussed for example in Luisi, 1998). For a criticism of this enterprise, see Cleland (2012).

[28] Examples are Cornish-Bowden (2006), Wolkenhauer and Hofmeyr (2007), Piedrafita et al. (2010), and Letelier et al. (2011).

[29] See Ruiz-Mirazo et al. (2014) for a review of the emerging field of Prebiotic Systems Chemistry and of the role played in it by definitions of life.

[30] The reason we emphasize *necessary,* rather than *sufficient* conditions, is that these are more pertinent tools in the scientific practices we examine here. The targets are simple life-like, prebiotic or minimal living systems, that is, systems

for the purposes of research, rather than sufficient[31]. The notion of *operational definitions* captures this use ─ which is dependent upon theory and directly involved in scientific practice ─ by pointing out the importance of the active (operational) role they play in the design and evaluation of observable operations in the relevant domain of investigation[32]. We agree with Fleischaker that in this context "the force of any operational definition is its capability of exhibition in the laboratory" (Fleischaker, 1990: 131). Accordingly, in our view, *operational definitions coherently combine, or integrate into a theoretical model, a set of mutually dependent necessary and satisficing criteria for life that imply observable operations, and that are considered pertinent and relevant for research*[33].

This approach fits with the way that many scientists, such as Luisi (e.g. Damiano & Luisi, 2011), refer to necessary and sufficient conditions for life but in the context of *revisable* or provisional definitions. Operational definitions provide a theoretical background to guide research and evaluate the relevance of experimental results, rather than strong demarcation criteria for a unique and rigid categorisation of natural systems.

As illustrated in Section 3.2, the principles for the use and experimental application of these definitions, i.e. criteria for *practical operationability*, can vary from one domain to the other: for example, design and construction of life for Synthetic Biology and Origins of Life, and guidelines for detection experiments in the case of Astrobiology. Criteria for *practical operationability* should not be confused with another important element of definitions, that is, *operational criteria* for life. The latter concern the specific contents of the definition, i.e., the set of relevant and pertinent necessary conditions for life according to a certain line of research. Consider for example the application of the definition of autopoiesis to the study of the origins of life. According to Fleischaker, "demonstration of the autopoietic criteria would result in the assembly of a living cell-system: a system boundary structure and a network of synthetic pathways for the production and replacement of system components" (Fleischaker, 1990: 131). The first part of the quotation concerns criteria for *practical operationability* indicating where, for what purposes, and how to apply the definition in the laboratory practice. In this case, the aim is to build a cell, in other cases, it may be to realise a simulation, to design tests to detect living systems, etc. The second part focuses on *operational criteria*: the specific contents of the definition that will be applied in a specific research practice. In this case, the contents include a boundary and minimal metabolism ("a network of synthetic pathways") capable of producing and replacing the components of the system.

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that do not exhibit all the features of life, or just the minimal ones. Accordingly, the focus of research is on individual, or sets of, necessary conditions for life, and on their emergence or precursors in the prebiotic world.

[31] We find that this use of definitions is better reflected by the term ‘satisficing’ rather than ‘sufficient’. The use of the term 'satisfice', a mix of 'satisfy' and ‘suffice', has been introduced by Herbert Simon (1956) to denote a heuristic strategy according to which a decision is made in real life when it satisfies the minimum requirements necessary to achieve a certain goal (see also Gigerenzer & Goldstein, 1996). It better fits our view of definitions of life, because the necessary conditions included in a definition reflect pragmatic choices that are dependent on practical and theoretical purposes. Moreover, this choice has a limited validity in time, insofar as definitions are refined in response to criticism, empirical results and new issues to be addressed.

[32] We refer to the use of *operational definitions* in the literature of these disciplines at the frontiers of Biology (see for example Fleischaker, 1990 and Luisi, 1998, among others).

[33] To make it clearer, definitions whose central properties and phenomena that are not *in principle* or practically possible to study in the laboratory or in simulations (e.g. entelechies or unspecified dispositions) do not satisfy the operational criteria.

The two dimensions do not necessarily coincide, as scientists sharing the same *operational criteria* might have different *criteria for practical operationability,* and apply the same definition in different ways and with different goals. For instance, while taking the same focus on boundary structure and synthetic pathways, Fleischaker (1990) aims at building a cell in the laboratory, whereas Agmon et al. (2016) aim at simulating the interplay between rates of metabolic reactions and changes in membrane properties.

### *4.1. The integrated nature of operational definitions*

The case of Luisi and collaborators shows that operational definitions are used and can be useful in science, not only for providing guidance to well-established research programs, but also for developing previously unexplored research lines and producing unexpected results. We do not claim that the cases examined are representative of all approaches to definitions in these fields. However, we take the examples to offer resistance to the view that attempts to define life are pointless. Our aim is simply to show that (*operational*) definitions of life *can be* useful in scientific practice, by guiding the development of a research program, providing criteria for the evaluation of results, and in generating new lines of investigations. Before we further unpack the virtues of operational definitions, we address the possible objection that the notion of definitions may not be necessary or useful to capture these aspects of the scientific practice.

Cleland and Chyba (2007), Griesemer (2015), and Bains (2014) propose to replace integrated definitions with a variety of "tentative criteria" or "practical tests" for life: a series of properties and phenomena usually related to our knowledge of life, but not defining or delimiting life. This approach offers a way to avoid the problems faced by definitions (in the traditional sense). However, the flexibility also implies a fragmentation of the criteria that results in difficulties of accounting for some important aspects of the scientific practices in which definitions of life are used. Specifically, tentative criteria cannot account for how life-processes are realized through organisms functioning as integrated wholes. A crucial aspect that distinguishes definitions from criteria for life in general is precisely that in definitions criteria are put together into a model or a set of *mutually dependent necessary conditions*.

An interesting aspect that can be evinced from Luisi’s work is that the use of an integrated definition of life allows far reaching investigations, such as those integrating metabolism and compartment. Inspired by Luisi's definition of life, Noireaux and Libchaber (2004) experimentally studied the integrated relations between metabolism and the permeability of the compartment. They emphasise how metabolism in protocells can improve the permeability of a lipid bilayer compartment. This process, in turn, improves the viability of metabolism itself by facilitating the exchange of biochemical compounds with the environment without lysing or perturbing the membrane. These integrative aspects may be missed if one focuses only on scattered criteria or properties of life.

The integration into one coherent system of several types of kinetic (catalysts), spatial (compartments) and template (genetic) components or subsystems is considered the basis for the realisation of a self-maintaining and self-producing biochemical machinery (see for example, Bich et al, 2016). Experimentally and theoretically speaking, this is not a trivial issue, but an extremely important one, because pre-existing molecular or supramolecular complexes cannot simply be recruited and coupled together. Integration requires a matching between the features of the subsystems involved: e.g., the composition of the membrane and the position of molecular machineries to meet the demands of metabolism, and the synthesis of the right components by metabolism to be used in compartments to achieve certain permeability (Shirt-Ediss, 2016). As recently argued by Alvaro Moreno, "the encapsulation of a self-maintaining chemical system has far-reaching organisational implications since its viability imposes significant changes on both parts (compartments and metabolic networks) in order to enable a functional coupling between them" (Moreno 2016, 10). The basic subsystems do not only need to be matched, but also their activity and rates must be functionally coordinated in order to achieve integration, and to avoid conflict.

Thus, a central aspect of defining life *operationally* is to investigate how the relevant conditions and processes included in definitions of life can be realised and made compatible with one another, in a theoretical as well as experimental sense. The distinct necessary conditions for life involved in a definition exhibit an integrated character that needs to be accounted for in theoretical, experimental and computational models representing a system as *living* or *life-like*[34]. A particularly hard challenge in the fields we examine is to integrate the functioning of different subsystems that are necessary to realise and maintain a protocell, a virtual cell, or a minimal living system in such a way as to achieve viable compatibility and interdependence (Rasmussen et al, 2008).

It may be objected to our proposal that examples of scientific practice fit better with the account of tentative criteria. For instance, Griesemer's alternative to definitions of life, centred on tentative criteria, is empirically based on an analysis of Gánti's chemoton theory (Gánti, 1975; 1979; 2003a; 2003b; Griesemer and Szathmary, 2009). Griesemer (2015) argues that despite using the term definition and proposing one, Gánti as an engineer engaged primarily in an explorative research program where criteria for life functioned as heuristics for the elaboration and manipulation of theoretical models. In our view, however, there is no contradiction between these two aspects (definitions and explorative heuristics). To explain why, we examine Gánti's work in further detail.

Gánti proposes a definition of life formulated in terms of a coherent set of criteria for life and an integrated model. His definition is based on ten criteria for life[35]: five absolute ones, to be satisfied by any living system (unity, metabolism, inherent stability, information carrying components, regulation) and five potential, necessary for the sustained existence of a living world (growth, multiplication, hereditary change, evolution, mortality). The absolute criteria are integrated into a model of a minimal living system capable of fulfilling them, in such a way that they are mutually dependent. The Chemoton (figure 4) is a hypothetical system organised as a biochemical "clockwork" (Gánti 2003b).

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[34] This is not necessary the case for all definitions. "Something is X if and only if it is red and square" does not raise problems of integration as long as 'red' and 'square' are independent properties. We thank an anonymous reviewer for pointing out the need to make this point more explicit.

[35] The criteria change slightly in different publications of Gánti's work.

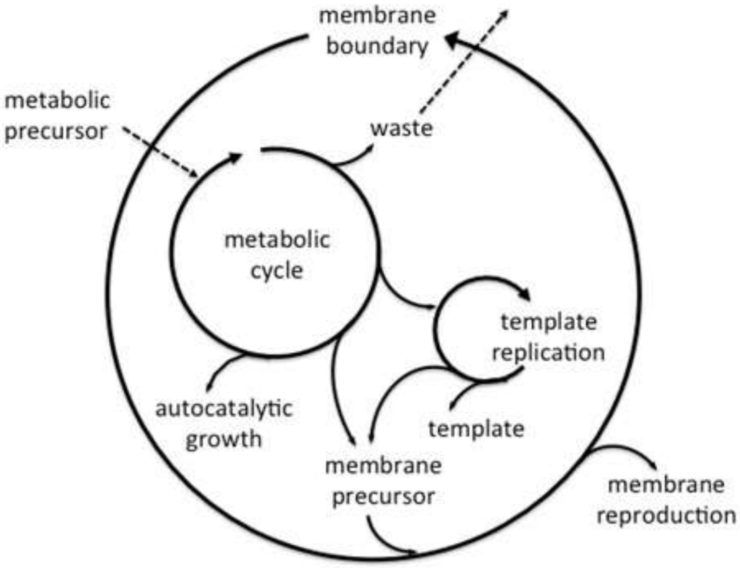

**Figure 4.** Scheme of Gánti's chemoton with the three coupled subsystems: a metabolic cycle, a template replication cycle and a compartment. Figure from (Moreno & Mossio, 2015: 26), with kind permission from Springer Science + Business Media.

Three autocatalytic reaction loops (metabolic cycle, template subsystem and compartment) are directly coupled to each other and realise the system as a unity. The individual unit (delimited by a membrane) is realised by the metabolic activity of production of the membrane and of the information carrying components. Metabolism, in turn, is made possible by the membrane, which allows substrates to enter the system, and waste to exit without accumulating in the systems, while avoiding at the same time the dispersion the molecules that are crucial for the internal processes. Metabolic components are also constrained by an information-carrying template (genetic) subsystem which, in Gánti's model in figure 4, specifies the possible length of metabolites. The different subsystems are then mutually stabilised by homeostatic mechanisms such as feedback loops and cycles. According to Gánti, the template also acts as an internal regulatory mechanism that "provides for the cooperation between genetic substance and homeostatic subsystem" (Gánti, 1979: 20) by controlling the system's functions and enabling the activity of the system as a unity, etc.[36] Gánti's Chemoton, therefore, constitutes a basic case of integration in which three of the most commonly mentioned necessary conditions of life, namely metabolism, membrane and template replication, are mutually dependent and functionally combined. Our view, thus, is compatible with Griesemer's interpretation of Gánti's goals without giving up (but accounting for) the effective use of definitions by Gánti and other theoretical biologists involved in a similar enterprise of building definitions as abstract theoretical models.

This dimension of definitions related to integration is especially important in our current historical context in which integrative approaches to life, such as that of Systems Biology, are being developed with the aim to overcome the intrinsic limitations exhibited by reductionist ones (Cornish-Bowden,

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[36] The characterisation of the template subsystem as a 'regulatory' mechanism is controversial, and it has been criticised by Bich et al. (2016).

2006; Wolkenhauer and Hofmeyr, 2007). As argued by Ruiz-Mirazo et al.: "theoretical and philosophical efforts to define life also contribute to those integrative approaches, providing a global theoretical framework that may help to deal with or interpret the huge amount of data being collected by current high-throughput technologies, in this so-called "omics' revolution"" (Ruiz-Mirazo et al., 2010: 203). Similarly, Fleischaker stresses the role of integration in a cautionary note on the study of individual mechanisms: "[A]lthough a particular mechanism might appear promising in that it satisfies one operational requirement or another, that mechanism is feasible for minimal cell operation only if it can be integrated with all the other operational mechanisms, it is theoretically consistent in combination. When we can provide the operational mechanisms and all their interrelationships in intricate detail, we will be describing a plausible candidate for the minimal cell" (Fleischaker 1990: 135-136). This point is still relevant in current Synthetic Biology, where the lack of clear theoretically grounded means of evaluating the outcomes of experimental efforts in building artificial cells has slowed the advancement of research in the field (Forlin et al. 2012).

In summary, while scattered criteria of life suggested by Cleland, Griesemer, and Bains avoid the criticism targeted towards strong ontological definitions, this approach misses one of the aspects that make living systems both interesting and difficult to study from the theoretical and practical points of view, namely their integrated character.

### *4.2 Further criticisms to operational definitions and responses*

To clarify further important aspects of operational definitions, we now address the last three of the general criticisms advanced by Cleland, targeted at the *use* of definitions. The first concerns how operational definitions relate to theories. Cleland (2012) argues that while definitions aim at capturing entire theories of life, theories are always wider and entail many more assumptions than what can be contained by definitions. This latter idea does not contrast with our view, according to which definitions summarize aspects of a theory that are considered crucial for a specific research project. They provide a set of necessary and satisficing (yet not sufficient, see footnotes 30 and 31) conditions for life, by selecting contextually relevant elements of the theory. For instance, Luisi's definition of life in terms of autopoiesis was built and used as a summary of the theory. Although reflecting only part of the overall theory, the definition was revised as a result of a shift within the theoretical perspective (to include responsiveness to the environment) and to enlarge it in response to new empirical research (from individual and isolated cells to cell colonies, and). Yet, from the operational point of view, unlike for Cleland, this is not a flaw: definitions play relevant roles in science due to the flexibility enabled by this very feature, because they facilitate communication, debate, and revision of experimental strategies.

The second criticism is that definitions might favour the entrenching of misconceptions, due to the fact that they tend to fix our knowledge into some static categories that make us blind to alternatives (Cleland, 2012). This problem is general for any heuristic strategy or model used in research. Yet, unguided and unbiased search for patterns or phenomena is rarely, if ever, a possible alternative. Operational definitions, like models or tentative criteria, are fallible tools that can be misleading but may also be used as stepping stones for better theories. The examples in Section 3.1 on minimal cognition and vesicle colonies show that insofar as definitions are theory based, they can be revised both within the context of a theory or as a result of revision of the theory itself. Whereas Cleland (2012) seems to assume that definitions of life aim (unsuccessfully) to express complete theories of life, we argue that definitions are instruments for the development of such theories. If theories are incomplete, provisional, and changeable, so or even more so are the definitions that summarise parts of them. Moreover, the very plurality of definitions of life, and the possibility of combining them (Oberholtzer, 1995; Szostak, 2001), suggest that these do not fix research and enforce of misconceptions but are continuously debated, challenged, and approached from different perspectives. In summary, agreement is not the pertinent requirement for a good definition.

Accordingly, we view the question about the importance of definitions of life as a matter of relevance and utility for research, rather than an issue about the potential for consensus[37]. For example, the lack of agreement on the definition of species does not prevent scientists from using this flexible notion to discuss extinctions and biodiversity (see for example Monastersky, 2014). Similarly, instead of seeing the existence of multiple gene concepts as an unwanted inconsistency in scientific terminology, multiple definitions can provide flexibility for distinct research purposes and insights to characteristics of different scientific fields or contexts (Waters, 2006). Importantly, the existence of disciplinary differences in concepts like ‘genes’ does not make it pointless for scientists and philosophers to reflect on limits and qualities of different definitions, or to discuss the type of gene concept that should be communicated to the public (Bartol 2013). The same is the case for disease classification where giving up the idea that diseases in general form a natural kind does not leave debates about the scope and content of disease concepts obsolete, e.g., as showed in discussions about how disease categories relate to abnormalities and risk factors (Scully, 2004). From this point of view, the very plurality of definitions used in biology is not pointless and useless, but can stimulate the debate and counteract the risk of dogmatic approaches to life and the entrenching of misconceptions.

This perspective also allows us to comment on Cleland’s proposed solution to the aforementioned problem of definitions in the context of Astrobiology, namely to focus on detecting *anomalies* rather than systems that satisfy a definition. In Cleland’s view, looking for anomalies allows for more flexibility than specific conditions for life selected according to a single, limited, framework. This is indeed an ingenious heuristic proposal, but is not as opposed to definitions as it might seem. It is also an operation that is theory dependent. Just like detection of abnormalities and diseases is dependent on knowledge about normal functional states, so is detection of abnormalities in this context dependent on an understanding of the 'normal' characteristics of life. In addition, approaches focused on anomalies, and more generally on *tentative criteria*, provide only tools to look for interesting cases, which are worth more attention. They do not provide *diagnostic tools* for discussing and deciding on

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[37] In the operational framework proposed here, the lack of consensus does not derive from a disagreement on how to demarcate life as a natural kind. Rather, it is related to the evaluation of different research programs (or subprograms) and modelling frameworks underlying definitions, i.e. it is a lack of agreement on which are the most relevant theoretical and practical problems to be solved and questions to be asked, and how to best address them. Disagreements on definitions are in this sense not different from scientific disagreements on the best model or modelling framework for solving scientific puzzles.

whether candidates for alternative life forms can be considered alive, which is what definitions help to do. If we accept an operational view of definitions, we believe that the two approaches should be considered as complementary, rather than mutually exclusive: a combination of negative heuristics based on the search for anomalies, and positive heuristics derived from tentative necessary and satisficing conditions of life according to instrumental definitions.

The third criticism draws on the argument that our current understanding of biology is still limited, and that we are far from having a general theory of living systems on which to rely to build general categorisations concerning life. From this standpoint, trying to fix our knowledge into definitions would obstruct science rather than contribute to its development. The main argument in support of this claim is that biology is based only on a single example of life: that part of the terrestrial biosphere which we currently know. Until we find other forms of life to compare to ours, we cannot distinguish essential from contingent properties of living systems and build a general theory of life (Cleland, 2012). Although the importance of discovering new forms of life is unquestionable, the assumption that encountering new life forms would possibly undermine our understanding of biology does not justify the need to suspend our attempts at developing and improving a theory of living systems. Science does not stop producing theories on the basis of the possibility that new discoveries might revolutionise them, and the idea of distinguishing sharply and ultimately between necessary and contingent aspects of the physical world has been problematised even in the context of physical laws (Mitchell, 2005).

Thus, there is no reason to abandon the project of a general theory of life, and stop thinking about what living systems are until we are certain to have the whole picture covered. Recall also that operational definitions allow for differences in practical operationability. The scope of definitions may vary depending on the purposes, and is not supposed to cover all possible scenarios. It is here interesting to note that Luisi (1998) criticises NASA’s definition of life (specifically the requirement of Darwinian evolution) as being too restrictive *in the context of Astrobiology*, while including some of its insights into his experimental program in Origins of Life (Oberholzer et al, 1995; Szostak et al, 2001). At the moment, however, it is not even clear whether or when we are going to find alternative life forms, and tentative criteria or anomalies alone provide limited help in their identification.

Finally, we should consider that life on Earth exhibits remarkable diversity with a variety of forms, behaviours and adaptability to different and extreme environments. Functionally speaking[38], therefore, a widely differentiated collection of examples is already available from which to start building well grounded, yet always provisional, theories and definitions capable of providing distinctions between some necessary and contingent properties of life and, also, to make sense of the increasing amount of data (Ruiz-Mirazo et al, 2010).

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[38] A functional perspective, open to multiple realisability in the molecular domain, can be generalised to other possible forms of life, as it is not univocally committed to the exact biochemical composition of life as we know it, that is: DNA, RNA and proteins made with the specific subset amino acids of known life, the same genetic code, etc.

## 5. Concluding remarks

We have argued that in order to account for the use of definitions of life in science, it is necessary to revisit the requirements we impose on definitions. In our view, at least two distinct and opposing kinds and uses of definitions of life are discussed in the literature. In this paper, we have contrasted what we can call strong definitions (Cleland, 2012) to our proposal of operational definitions (Fleischaker, 1990; Luisi, 1998)[39]. Strong definitions aim at providing answers to questions regarding natural kinds, by specifying necessary and sufficient conditions. As we demonstrated with examples, operational definitions better capture the use of definitions in Origins of Life and Synthetic Biology. These are considered tools that express and integrate necessary criteria for life that are considered pertinent, relevant and satisficing for research, and that imply observable operations.

These two kinds of definitions are characterised by different and often incompatible claims, requirements, and aims (Figure 5). Strong definitions attempt to provide a complete set of necessary and sufficient conditions. In contrast, operational definitions focus on an open-ended set of possible necessary and satisficing conditions for life according to the specific requirements and goals of different research programs. Accordingly, while strong definitions might establish static categorisations, operational ones allow for flexibility and revisability. Finally, whereas strong definitions strive for unification and thereby set consensus as their success criterion, operational definitions rely on pluralism as a source of production of novel knowledge, and on practical and theoretical utility as the featured value. While the former are more demanding in terms of philosophical justification and are subject to profound criticisms (Machery, 2012; Cleland, 2012), the latter better conform to scientific practice and goals, to the actual uses of definitions in science, and can be applied to specific case studies.

| STRONG DEFINITIONS | OPERATIONAL DEFINITIONS |
|---|---|
| **(Strong) ontological claim**<br>*Demarcating life: carving out natural kinds* | **Instrumental claim**<br>*Theoretical and epistemic tools: guidance for debate and experiments* |
| **Complete**<br>*Necessary and sufficient conditions* | **Open-ended**<br>*Necessary (and satisficing) provisional conditions* |
| **Fixed**<br>*Static categories* | **Flexible**<br>*Variation in practical operationability*<br>*Definitions evolve over time* |

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[39] Strong and operational definitions are not the only possible kinds of definitions of life. Intermediate positions between these two are also possible, for example combining instrumental claims with more moderate ontological ones (an example is Ruiz-Mirazo et al., 2004), which nevertheless would require a philosophical justification against Machery's and Cleland's criticisms.

| | |
|---|---|
| | |
| **Unification**<br>*Consensus and generalization of characteristics of all life are the key values* | **Pluralist**<br>*Target of debate, challenge and revision*<br>*Utility is the key value* |

**Figure 5**. Comparison between strong and operational definitions

In summary, we have questioned the assumption that the only (or even main) purpose of definitions of life is to establish a set of universal criteria that strongly demarcate natural kinds. In doing so, we have focused on the role played by definitions in scientific practice rather than on whether or not strong ontological definitions are possible. From this standpoint, we suggest that the use of definitions in scientific practice may be better captured by a pluralist and operational perspective in which definitions can serve various epistemic purposes by combining variety and flexibility with the need for useful tools and categorisations towards scientific aims. Even if the criticisms against strong definitions of life are accepted, there is still a role for definitions of life in science, which is played by *operational* definitions. We have highlighted how a number of case studies demonstrate the practical and theoretical value of this kind of definitions in guiding the classification, detection, design, and understanding of different life forms.

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