A roadmap towards the synthesis of Life.

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Abstract

The synthesis of life from non-living matter has captivated scientists for centuries. It is a grand challenge aimed at unraveling the fundamental principles of life and leveraging its unique features, such as resilience, sustainability, and the ability to evolve. Synthetic life holds immense potential in biotechnology, medicine, and materials science. Advancements in synthetic biology, systems chemistry, and biophysics have brought us closer to achieving this ambitious goal. Researchers have successfully assembled cellular components and synthesized biomimetic hardware for synthetic cells, while chemical reaction networks have demonstrated potential for Darwinian evolution. However, numerous challenges persist, including defining terminology and objectives, interdisciplinary collaboration, and addressing ethical aspects and public concerns. Our perspective offers a roadmap toward the engineering of life based on discussions during a two-week workshop with scientists from around the globe.

Introduction and Motivation

The synthesis of life encompasses the creation of living organisms from non-living matter and has captivated scientific curiosity. It is driven by the pursuit of unraveling the fundamental principles of life and the prospect of developing innovative life forms harnessed for practical purposes. Decades of research in synthetic biology, systems chemistry, origins of life, biophysics, bionanotechnology have brought us closer than ever to synthetic life. [1-7] For example, functional cellular machinery for transcription and translation has been assembled inside lipid vesicles.[8] Concomitantly, biomimetic hardware has been synthesized for synthetic cells using protein engineering[9] and DNA or RNA nanotechnology.[10] In systems chemistry, chemical reaction networks that contain exclusively non-biological molecules have recently shown some elements of Darwinian evolution.[6,11-14] Yet, significant technical and conceptual challenges remain. One major challenge to the synthesis of life is the lack of clarity on the goals. We must abate the lack of consensus on the definition of life and other important terms. Moreover, synthetic life does not only concern scientists—the perception by the public, media, and politicians, the investments by funding bodies, and the interest by publishers play a role too.

Despite the challenges, synthetic life has far-reaching potential in academia and industry. By synthesizing life, scientists aim to learn about what life is, its minimum requirements and emergence, and what would be needed for its existence in niches beyond Earth. But, beyond these fundamental questions, synthetic life is motivated by its practical benefits, be it in biotechnology, medicine, or materials science. Synthetic cells can be used as biofactories to produce valuable pharmaceutical products, degrade pollutants, convert and store energy, or capture greenhouse gases.[4,15] Because they can be evolved to perform one specific task, they can surpass the efficiencies of natural cells. Evolving synthetic cells will further accelerate the rapid growth of the global SynBio market.[16] Particularly powerful in such materials is the concept of "evolving materials"— materials that follow the principles of Darwinian evolution to find the fittest solution for problems presented by its designer. Directed evolution has been groundbreaking for developing new enzymes, giving a first glimpse into the vast space of opportunity for the evolution of synthetic cells. Moreover, given that evolution tends to come up with multiple solutions to selection pressure, it can be expected that evolving materials result in more robust solutions than classical materials design. Finally, even before the end goal of the synthesis of life has been reached, we expect to see technological advances that result from the research, for example, in gene assembly like Gibson assembly[17,18], high-throughput selection, and information encoding and sequencing of non-biological heteropolymers. Given its broad implications, in this perspective, we offer a cross-disciplinary roadmap toward the synthesis of life that discusses the field's progress, motivation, terminology, ethical concerns, and challenges. These have been compiled after a two-week workshop with 57 scientists from 14 countries (see author contributions).

1. What is the overarching goal. Engineering synthetic life needs specific, identifiable targets and milestones to measure the progress of the field, which would, one way or another, require a definition of life. While many definitions have been proposed,[19,20] no overarching definition has been agreed upon. Instead of attempting to define life, we could identify a list of hallmarks, such as metabolism, compartmentalization, replication, motility, and response to stimuli, and try to incorporate these into non-living systems. These definitions tempt us to realize one hallmark after another and leave their integration until the end. This approach leads to exciting systems with "life-like" traits and has already yielded important insights into the workings of biology and biomaterial design.[21] But the collective organization of such hallmarks may not give us synthetic life. Yet, because life is not modular in a technical sense, synthesizing a system that contains some of life's hallmarks may not give us synthetic life—for instance, a motile compartment that can divide and has a metabolic reaction network does not qualify as synthetic life. Instead, we should aim for an autonomous chemical system that can undergo open-ended evolution. In such a system, we expect life-like features like those listed above to emerge naturally.[20] Put differently, hallmarks such as responsiveness or motility control are evolvable functions that can be expected to emerge spontaneously once one has achieved open-ended evolution.

Thus, we can define our target as synthesizing a **self-sustaining chemical system from non-living matter capable of open-ended evolution**, an adaptation from the "classical NASA" definition of life" (Fig. 1a).[6,7,22-24] A *chemical system* implies we are dealing with molecules in a chemical reaction network, which sets this field apart from *artificial life*, which includes life in non-chemical settings like in silico synthetic life or synthetic life based on robots. Life is *self-sustaining*—it continues to operate with precursors, building blocks, and energy offered by the environment. *Metabolism* is responsible for self-sustainment: environmental precursor molecules are autonomously converted into building blocks for life. Moreover, synthetic life should be synthesized from non-living matter. That means that the building blocks that the self-sustaining system needs cannot be based on living systems. That does not mean life cannot be synthesized from biologically derived molecules like DNA and reconstituted or purified proteins or dead cells.

Synthetic life has to evolve following the principles of biological *Darwinian or Lamarckian evolution*. That means that the living entity must be able to replicate—it must autonomously make a copy of itself. At the most primitive level, that can involve the conversion of non-replicating molecular precursors into replicating molecules. At a more complex level, a living entity must copy its information-storing substrate, *i.e.*, its genotype, and ensure all other critical components, such as a new compartment and catalysts for metabolism, are also self-synthetized. The copying process will yield mutations in the genotype of the self-replicating systems propagated to the next generations. These mutations must affect the phenotype. As self-replicating systems compete for resources, the fitter mutants will thrive at the expense of others—the principle of natural selection. Natural selection implies that self-replicating systems can decay, especially when resources are scarce. Decay makes their building blocks available for competing self-replicating systems. Finally, Darwinian evolution should be open-ended, *i.e.*, random mutations will lead to a vast, practically infinite set of possible genotypes. Only a small subset of these possible replicator genotypes is realized at any given time. Open-ended evolution makes the present replicating systems move through this sequence space and "explore" the fitter genotypes (Fig. 1b). Under those conditions, one can expect a never-ending evolution yielding surprising solutions, including the evolvable, life-like list of hallmarks mentioned above.

The breadth of the target we identified offers a broad choice of the building blocks to synthesize life—from biological hardware to simple, synthetic molecules and anything in between. The choice of building blocks does affect the research questions and applications that can be tackled. For example, synthetic life based on simple, prebiotically plausible molecules can help unveil minimal mechanisms that operate at the origins of life. In contrast, synthetic life built with highly evolved biomolecular machinery will be powerful in creating biofactories.

Taken together, we aim for a minimal entity capable of open-ended evolution with a wide range of molecular building blocks at our disposal. Given that a cell is seen as the minimal entity of biological life, we could call such a minimal entity a synthetic cell. The term synthetic cell is frequently used in the bottom-up synthetic biology community to describe systems that exhibit features of life but are not yet alive.[3] Instead, we aim for a living version of a synthetic cell, *i.e.*, a "living synthetic cell". Our workshop has shown that members of the systems chemistry community do not yet feel included when discussing synthetic cell research because the term cell feels restrictive to the use of biological building blocks only. Therefore, we use the term synthetic life to define our aim broadly. "Synthetic life" serves as an umbrella term for the terms "synthetic cell", "artificial cell" or "protocell" more frequently used in synthetic biology and the Origins of Life, and "de novo life," which is often used in systems chemistry (see Glossary in Box 1).

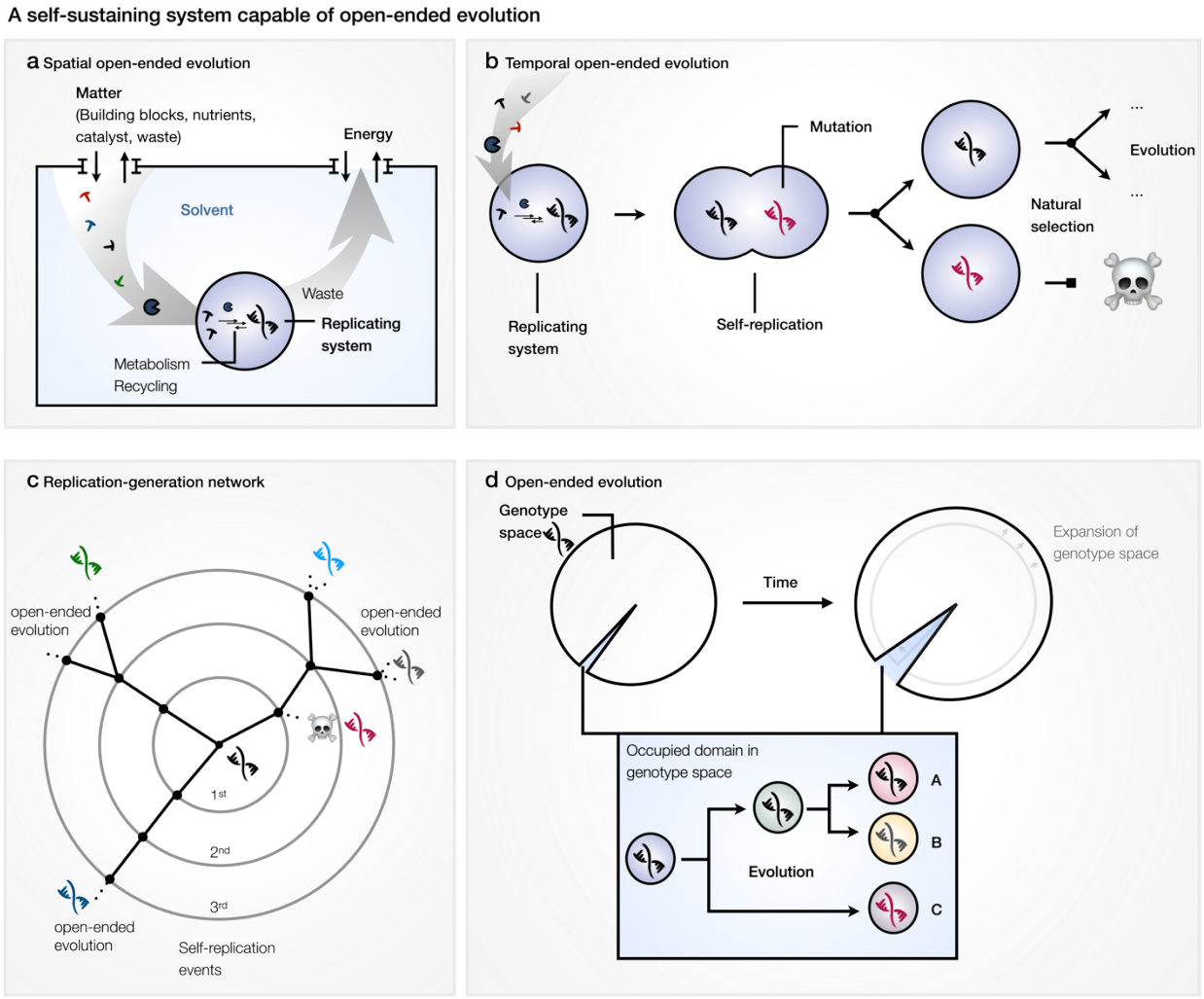

Figure 1. The overarching goal: synthetic life from non-living building blocks. a-b) *Life is depicted as a self-sustaining system. Energy and nutrients are supplied into an environment. Life uses resources to replicate and sustain itself. Mutations in the replication process result in diversity in the genotypes and phenotypes. Through natural selection, fitter mutants thrive while the weaker ones decay.* ***c-d)*** *Natural selection can result in open-ended Darwinian evolution when a vast, practically infinite phenotype space is available, but only a tiny subset is occupied. That way, evolution can continuously explore new phenotypes and new environments without end.*

2. The state of the art. There has been considerable progress toward the individual prerequisites for the synthesis of life from various disciplines, including systems chemistry, biophysics, synthetic biology, DNA/RNA nanotechnology, and others.[1-7] Each of their contributions shares a common goal separated by the choice of molecular building blocks and environments. We discuss the state-of-the-art, starting from natural building blocks and progressing to increasingly more synthetic hardware (Fig. 2). The aim is not to offer a comprehensive overview of the literature but to identify critical developments from different fields.

The most obvious way to synthesize life may be to start with a preexisting cell. In 2010, a synthetic cell was made by synthesizing a minimal genome and inserting it into host cells whose original genetic material was removed. The engineered cell is governed by a synthetic genome and can reproduce.[25] Today's version of this cell, JCVI-syn3.0, has as little as 473 genes[26]—some are

involved in crucial processes, such as transcription and translation, others with unknown but critical functions.[27] This top-down assembled version of a synthetic cell is an impressive example of the generation of new forms of life from life. Still, all molecular building blocks apart from the genome were assembled by living cells. There are ongoing efforts to boot the minimal genome inside a synthetic compartment[1,28] containing all components to start the transcription and translation processes. Once successful, this would be one route toward the synthesis of life.[29,30]

Bottom-up synthetic biology aims at yet simpler versions of synthetic cells and constructs these based on separate and increasingly basic components. Lipid vesicles and other compartments have been equipped with cell-like functionality by encapsulating minimal sets of proteins. In this way, specific hallmarks of life, such as energy conversion,[31,32] could be implemented, yet machinery for self-regeneration still needs to be added. Thus, much of the community focuses on *in vitro* transcription-translation to produce functional sets of proteins inside the compartment instead of encapsulating pre-synthesized ones.[1,8,33-36] However, it remains a great challenge to self-replicate all necessary components.[37] As an intermediate strategy, these components can be supplied from the environment. Around 200 genes are estimated to be required for a simple self-regenerating system.[35] Thus, the top-down and the bottom-up approaches may converge at some point, yielding a minimal self-replicating set of genes.

This leaves room to ask whether engineering solutions can further simplify life. One strategy is to engineer peptides and proteins to accomplish division and regrowth cycles based on fewer, simpler components. For instance, the encoding of the production of compartment-forming peptides genetically was demonstrated.[38] Still, in such an approach, the many components to transcribe and translate these peptides are still required, which would need to be replicated when such a compartment self-replicates. Thus, attempts have been made to engineer functional molecular hardware directly from DNA or RNA. Intricate DNA origami structures have been used to mimic transmembrane proteins[39], cytoskeletal filaments[40], or compartments[41,42]. In such a strategy, information and function use the same molecule (DNA), disregarding the need for complex transcription machinery. Noteworthy, by taking this shortcut, the genotype-phenotype separation is lost—the genotype becomes the phenotype.[43,44] Recent progress on the co-transcriptional folding of RNA origami[45] enables the genetic encoding of such structures while avoiding the entire translation machinery, as long as polymerases are supplied from the environment.

Fully self-replicating systems are available when we allow simplifying building blocks even further. Systems chemistry has seen a rapid increase in chemical systems capable of replicating themselves without using complex biological machinery.[24,46] For example, DNA has been demonstrated to replicate using non-natural, chemically activated nucleotides.[47,48] DNA origami has yielded information encoding and copying structures where information is not directly encoded in the DNA bases[49]. Ideas have been put forward on the self-replication and evolution of DNA crystals[50]. Besides DNA, self-replicators based on RNA,[51,52] peptides,[53] and non-biological building blocks exist.[46,54-62] The beauty of these systems is that the genotype is replicating itself, negating the need for complex replication machinery.[54] Excitingly, mutations in such self-replicating genotypes have recently been demonstrated, opening the door to Darwinian evolution.[63] Moreover, self-replicating molecules can catalyze reactions besides replication, which allows for metabolic reaction networks needed for open-ended evolution.[24] Recent work has shown that self-replicating stacks of macrocycles can catalyze other chemical reactions besides their formation.[12,64] When combined with years of work on using chemical reaction networks to regulate molecular self-assembly[13,21,55,65-77], it opens the door to a catalytically active genotype also regulating its environment.[78]

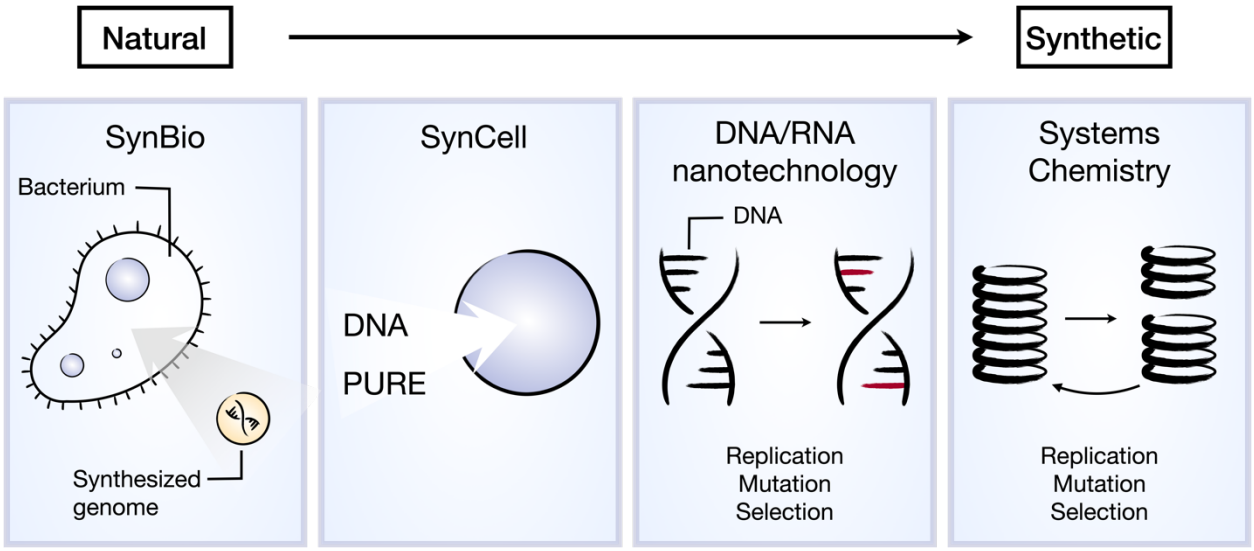

Figure 2. The current state of the art in synthetic life. *Systems differ in the chosen set of building blocks, from natural to synthetic. Synthetic life encompasses life engineered based on biological components (left) and chemically made life (right).*

3. What are the challenges ahead?

Despite the progress in the field, several outstanding challenges need to be addressed. We have identified ten key challenges—some are more technical and therefore system-dependent, whereas others affect us all.

Unifying our community through a common language. The endeavor to synthesize life attracts scientists from diverse disciplines, including classical biology, systems chemistry, DNA/RNA nanotechnology, and biophysics. Therefore, effective communication requires a commonly understood language. Biologists have studied life for centuries. They developed a well-established language to describe its concepts, mechanisms, building blocks, and properties. Historically, these terms were only relevant for life as we know it and, by extension, for biological building blocks. For instance, in biology, Darwinian evolution implies mutations in an organism's DNA, which can increase or decrease its fitness. Genotype-phenotype-mapping[79] relies on gene expression according to the central dogma of molecular biology. Therefore, Darwinian evolution, genotype, and phenotype are tightly related to a limited set of biomolecules. As discussed, synthetic life should rely on these principles, but does this imply that the use of biological language is questionable? Are terms like cell, genotype, phenotype, and Darwinian evolution reserved for biology, or can we apply them to synthetic life, too?

We advocate for the latter as it is challenging to agree upon new terms and consistently use them. Synthetic life should be described with biological terms as long as they are understood more abstractly and inclusively. This requires a continuous effort by all members of this young community: we cannot just assume that our more generalized use of biological terms is understood and accepted immediately by all. Therefore, we should highlight the generalization whenever we use a biological term to describe a system that is not based on biological building blocks. Similarly, we must remain self-critical and highlight the limitations of the analogies we draw. We need to take care that the terms first coined by biologists are not used to oversell results or in a way that is no longer consistent with their original meaning. Carefully done, the abstraction of language can help move forward biological research and the synthesis of life. We present a few of such generalizations in the Glossary in Box 1. This is not the complete set of terms and

should be continuously discussed in, for example, review articles, papers, and interdisciplinary workshops.

Communication with the public. Besides communicating effectively within the community, we must also communicate with the public. The synthesis of life can be perceived as “playing god” or ‘Too high goals' and 'megalomaniac’ if the ethical and moral considerations taken by the researchers are not transparently communicated. Another concern is that sensationalization of synthetic life can instill fear in the public and attract undesirable attention, which can be avoided by avoiding overclaiming or exaggeration. Therefore, synthetic life research must not be oversold but communicated as facts and their reasonable implications. Future technologies can only be successfully translated if the scientific community is open about the risks and opportunities these technologies provide. Therefore, the synthetic life community must regard science communication as a central, essential effort to achieve maximal transparency and, ultimately, an acceptance of a new manufacturing paradigm.

Conventional measures of science communication often only reach groups with a high level of prior information. Therefore, a ~~s~~ynthetic life communication strategy must be crafted to reach harder-to-reach target groups, *e.g.*, individuals with a lower educational background. Consulting social science experts, our community seeks strategies for scalable, inclusive, and two-way science communication. In particular, we advocate for bottom-up science communication measures, which can easily be integrated into day-to-day research, such as web video conferences (see, *e.g.*, www.ring-a-scientist.org). Schools are good target groups because they allow us to broadly reach society’s next generation. After all, we are dealing with topics that have sparked humanity's curiosity for millennia.

Establishing interoperability. For synthetic life, a set of building blocks and an environment capable of open-ended evolution must be chosen that does it all. That means all chosen building blocks must act interoperably to achieve the minimum requirements for life. This approach may contrast the classical approach of designing separate life-like features into different non-living systems. Nevertheless, establishing interoperability does not mean that the community has to agree on a molecular canvas for synthetic life—we should not aim for a single form of *de novo* life. Different chemical systems may give rise to different new life forms, for example, one based on nature’s building blocks and transcription and translation machinery; others may use engineered hardware or entirely synthetic building blocks (Fig. 2). A multidisciplinary approach is a strength of our attempt to join forces in the synthesis of life as it increases the chances of success and the range of possible future applications.

Synthesizing a self-sustaining chemical system. Life is self-sustaining—autonomously sustaining and replicating itself using energy and building blocks from its environment. Thus, choosing the right environment and the method to maintain an open system are essential. One should consider the inflow of building blocks, such as simple molecules, enzymes, or even cell lysates, and energy carriers, such as high-energy reagents (fuels or nutrients), light, or temperature gradients. A challenge in choosing a suitable energy carrier is to find replicating systems that convert enough energy from their surrounding. If the energy conversion rate is low, replication is slow, and related non-equilibrium phenomena such as force generation are negligible.[80] At higher energy conversion, more non-equilibrium states can be accessed that provide a natural selection pressure once the speed of evolving new genotypes is favorable. This trend is naturally competitive as resources provided by the outside are limited. Finally, the accumulation of waste often arrests chemical turnover, preventing further evolution. However, if a system undergoes open-ended evolution, it may intrinsically develop mechanisms to degrade and reuse the waste.

Designing degradation-and-reuse pathways. In biology, death is an organism's irreversible decay—a critical component of natural selection. Without death, species replicate exponentially until all resources are consumed, and no more open-ended evolution can occur. Similarly, in synthetic life without decay, once all resources are consumed, no further selection can take place. A significant milestone remains the implementation of decay and recycling mechanisms for the progress in the synthesis of life. Like biology, this could result in the irreversible decay of the replicating system that renders its building blocks available for competition. Such decay mechanisms could be designed using chemically fueled assembling and replicating systems.[81] Parasitic behavior has also been explored in which one self-replicating system depends on the building block of another.[82]

Alternative approaches exist in which selection can occur without decay mechanisms. For example, in serial transfer, self-replicating systems compete for a finite pool of resources. After some time, a small amount of the solution is transferred to a new solution of resources. Repeated replication-transfer steps will select replicators that produce sufficient offspring to ensure that at least one replicator is transferred to the next pool of resources.[82] A challenge with such alternative decay mechanisms is that they select for the fastest replicator, colloquially referred to as Spiegelman's monster[83]. Creative methods involve compartmentalization to prevent a takeover by the faster replicating molecular parasites,[84] but ultimately, chemical degradation pathways are likely critical for open-ended evolution.

Coupling genotype to phenotype. For life as we know it, the genotype-phenotype coupling is established *via* the transcription-translation machinery known as the central dogma—DNA is transcribed into RNA, which is translated into proteins. For synthetic life, the genotype does not necessarily refer to a given sequence of bases in the DNA but, more generally, to the system's information content, which is replicated (see Glossary in Box 1). We can challenge the necessity of genotype-phenotype coupling through the transcription-translation machinery for synthetic life. While it is perfectly valid to use *in vitro* transcription-translation systems in synthetic life, it is at least conceivable that synthetic life uses only transcription or, more radically, that a single molecular entity confers genotype and phenotype, *i.e.*, it has a certain sequence with endows it with a certain conformation. Separating genotype and phenotype is desirable as it boosts the system's capacity to evolve but may not be a prerequisite for life per se.

For synthetic life that relies on the central dogma, replicating or harvesting the entire transcription and translation machinery from the environment is challenging. Therefore, new mechanisms not relying on the central dogma for genotype-phenotype coupling should be designed for synthetic life.[85] For example, the information-encoding molecules could perform functions besides carrying information. For example, self-replicating RNA systems with limited complexity function as the genetic material, structural component, and catalyst.[86] Noteworthy, the idea that an information-encoding molecule performs functions besides carrying information is also the basis of the RNA world hypothesis for the origins of life. Besides RNA, a staple-strand sequence encodes the information for the final geometry in DNA origami. Thus, the DNA (genotype) also encodes the shape of the assembly (phenotype), similarly, for completely non-biomolecular self-replicating systems such as the self-replicating molecular stacks of macrocycles[12,64] described in the state-of-the-art.

Even when genotype and phenotype are coupled, challenges exist. While selection remains the driver of phenotypic change, the significance of genotype-phenotype coupling has become increasingly obvious. Characteristics such as the landscape of the genotype space, the heterogeneity of the environment, or the probability of lateral gene transfer can strongly determine evolutionary outcomes. Therefore, we need more than a simple link between genotype and phenotype for open-ended evolution. The properties of natural genotype-phenotype maps have

been studied extensively, resulting in several models replicating their properties and testing evolutionary trajectories in silico.[79,87] Suitable genotype-phenotype maps have to fulfill a set of properties that are essential for their evolvability[79]:

1) Redundancy: Multiple genotypes map to the same phenotype. Without redundancy, evolutionary processes would never find viable phenotypes in the vast space of possible sequences.
2) Bias: Some phenotypes are represented by many genotypes, while others are encoded only by a few.
3) Robustness: A certain fraction of possible mutations leave the phenotype unchanged. More drastically, significant changes in the genotype frequently have no impact on the phenotype. Robustness seems to oppose evolvability, yet it has been shown that one can benefit the other on the phenotype level.

Tuning mutation rates. Darwinian evolution is impossible without mutations in the genotype, which allows life to move along the fitness landscape. Nevertheless, too-high mutation rates make adaptation impossible, whereas too-low mutation rates mean that open-ended evolution cannot occur on experimentally accessible time scales. Thus, there is a delicate balance between stability and evolvability, described as the critical mutation rate.[88] The critical mutation rate, or error threshold, refers to the number of bits, *i.e.,* the number of base pairs in a biological cell, that a self-replicating molecule may have before mutation destroys the information in subsequent generations of the molecule. In the origins of life field, Eigen's paradox[89] describes the unsolved puzzle of how sufficiently long DNA sequences could be copied faithfully enough without error-correcting enzymes. On the other hand, small genomes or systems built on synthetic chemistry may suffer from the opposite problem, *i.e.*, the need for more diversity that they can generate and lead to their growth.

For DNA/RNA-encoded synthetic life, methods developed in directed evolution can be used to initially fine-tune mutation rates by designing appropriate DNA libraries. For the system to tune mutation rates by itself, it is possible to use DNA polymerases with appropriate error rates, like the Taq polymerase[90], and to increase mutations further, for instance, by using manganese ions[91] or other physical and chemical factors[92,93]. If synthetic information-encoding molecules are used, strategies to tame mutations must be developed.

Establishing open-ended evolution. Self-replicating systems have demonstrated Darwinian evolution in rudimentary form, yet open-ended evolution remains a challenge. We must identify self-replicating and evolving systems that can exhibit unbounded growth in complexity (see Glossary in Box 1). Moreover, a vast number of possible genotypes is required, such that the system occupies only a tiny fraction of possible phenotypes in the genotype-phenotype space at any given time (Fig. 1d). It is crucial to develop a quantitative understanding of the critical mutation rates for each system and genome size to tune mutation rates such that open-ended evolution can occur on experimentally accessible time scales. While established theories have been proposed on the requirements and measures for open-ended evolution,[94,95] it is a significant challenge to implement those in synthetic systems. As such theories are based on general principles, open-ended evolution can be realized with different sets of molecular hardware, which allows for diverse approaches towards the synthesis of life as discussed in this perspective.

Quantifying our progress. How can we quantify our progress toward synthetic life? Is it a sudden transition from a non-living to a living system or a smooth process in which a system increases its liveness? We propose two approaches that differ in the quantifiers for life.

In the first approach, the system is scored by quantifiers describing the fundamental prerequisites of life (see List of Hallmarks in Box 2). That means a vesicle with a self-replicating genotype is

further from life than one with a self-replicating and mutating genotype. Such qualifiers can be further quantified, for example, by scoring replication rates and fidelity. Consequently, a non-zero value for this score does not imply that a system is living, and there is no threshold for transitioning from a living to a non-living system.

As a second approach, we propose using quantifiers for evolution with a particular focus on its open-endedness, for example, by measuring the information content of genotypes or correlation functions for spatial patterns of the genotypes. Moreover, the progress of evolution can be characterized through the realized phenotypes (frequency, function). Solving this challenge allows for characterizing evolution and scrutinizing its open-endedness through genotype-phenotype maps. Such maps provide the basis to unravel the respective couplings and provide on the system's robustness, redundancy, and bias using established methods.[79]

Establishing ethical considerations. Ethical considerations regarding the risks, accountability, responsibility, the value of life, and public perception must be evaluated and continuously re-evaluated when synthesizing life. Arguably, the most significant risk is that synthetic life might escape containment and interact adversely with natural ecosystems. Authorities have begun to realize that we need safety procedures, like those instated in biological sciences, to prevent the escape of genetically modified organisms for synthetic life (*e.g.*, dedicated synthetic life labs with containment procedures).[96] This implementation further complicates when synthetic life is used in materials, such as a synthetic cell used as a biomaterial. Here, the field must adapt already existing procedures for such materials. Ideally, it is controlled by international organizations like iGem for synthetic cell research, which has already been demonstrated (https://responsibility.igem.org/). These measures are implemented for well-intentioned scientists, but risk mitigation strategies should also be implemented. As an analogy, the field of organic chemistry permitted the development of modern medicine and saved countless lives, but it also led to the development of chemical weapons. Biosecurity risk mitigation strategies must be implemented now, while synthetic life is still in its infancy, to avoid its misuse and prepare against it.

More complex are questions regarding responsibility and the value of synthetic life. Who is responsible for the actions of synthesized life forms? How do we ensure that these forms of life are treated ethically? How evolved does life need to be to obtain rights? These questions must be carefully considered and constantly reconsidered as the field develops.

4. Conclusions

The synthesis of life is fascinating as a fundamental endeavor and for its endless potential. Already, synthetic cells help us understand the biochemistry and biophysics of life and its origin. Besides, synthetic cells are leveraged to synthesize valuable pharmaceuticals and proteins. We foresee that synthetic life can further push those existing applications and open new avenues. For example, it can revolutionize how we develop materials from the classical design and engineering approach to an evolving approach. Catalyst development has already gone through this revolution with the onset of directed evolution, showing us a glimpse of the possibilities.

This perspective offers a roadmap to aid the synthesis of life by discussing the state of the art and setting out ten foreseeable technical and non-technical challenges ahead. The technical challenges mainly concern establishing a self-sustaining and mutating system capable of open-ended evolution. We strongly advocate for interoperability—one system must do it all. On the non-technical side, we identify that communication is critical—be it within the community or the general public. Some view life as something sacred that should not be altered. Clear communication on what we do and why we do it is critical when pursuing this field. We strongly advocate using a common language to facilitate interdisciplinary communication. The synthesis of life is a

multidisciplinary field aiming for the same goal, separated only by the building blocks we chose to work with. We established such a language by defining terms in the Glossary in Box 1. The challenges we identified are far from complete and not set in stone. We anticipate that, as the field develops, new challenges will arrive. Moreover, non-technical challenges like ethical concerns and effective communication with the public affect us all and should remain continuously debated.

The synthesis of life is a fascinating and rewarding endeavor. Given the scientific interest, we are optimistic that synthetic life is achievable from all of these disciplines in the coming decades. But there are massive challenges ahead and more to come.

Box *1 Glossary.*

Replicating system *is a set of chemical components that makes copies of itself. Replication can be enabled by molecular machinery that is part of the environment or through self-replication, corresponding to making autonomous copies of oneself. A replicating system can be as simple as a single type of molecule (a self-replicator) or a complex set of chemical components (e.g., a cell).*[6]

The environment of a replicating system *constitutes the chemical conditions (such as the solvent, precursor molecules, temperature, buffer capacity, and pH) for the replicating system. The replicating system's environment is an open system that exchanges energy and mass with the outside. For synthetic life, none of the environmental components are alive.*

Open chemical systems *are mixtures composed of chemically reacting components that can exchange energy and matter with a reservoir. While the open system hardly affects the reservoir, energy and matter exchange with the reservoir can maintain the open system away from thermodynamic equilibrium.*[97]

Metabolism *is a chemical reaction network that builds the compounds needed for the self-sustainment and replication of a living system from simpler chemicals.*

A self-sustaining chemical system *is a chemical system that can regenerate all of its system's components and does not require continuous intervention by a higher entity, such as us scientists.*[22]

Mutation *refers to a stochastic alteration in the genotype of the replicating system that is more or less permanent and can thus be transmitted to the descendants. These changes can occur during replication or due to external perturbations (e.g., light, reagents, and radioactivity). A prominent example is the changes in the sequence of heteropolymers such as DNA and RNA.*[6] *In systems chemistry, an example is mutations in the self-replicating stacks described by Otto et al.*[11]

In biology, the ***genotype*** *of an organism is defined as its complete set of genetic material, i.e., the information needed for the construction of the organism. We propose defining the genotype in synthetic life as the information needed to construct the replicating system. This information can correspond to the self-replicating stacks described by Otto et al.*[54]*, or the DNA in self-replicating synthetic cells.*

In biology, the ***phenotype*** *is defined as an organism's observable characteristics or traits. We define the phenotype in synthetic life as all extra properties that the system obtains beyond the information needed to construct the replicating system (genotype). This can*

be as simple as a self-replicating RNA's ability to fold, phase separate, and catalyze reactions other than its replication (i.e., the RNA-world hypothesis) or as complex as the translation-transcription machinery synthesizing functional proteins to form higher order assemblies (i.e., the central dogma).

***Darwinian evolution** is the process of changing genotypes through the natural selection of a fitter phenotype, i.e., the individual's ability to compete, survive, and reproduce. Mutations in the genotype occur randomly through environmental influences. The fittest mutants survive.*

***Lamarckian evolution** is similar to Darwinian evolution except for the mutation process. In Lamarckian evolution, the adaptation process is a direct response to mutations induced by the environment. Those adaptations are passed on to the next generation. In contrast, in Darwinian evolution, mutations are entirely random, leading to survival of the fittest.*

***Open-ended Darwinian evolution** occurs when the **genotype and phenotype** steadily change over time and show an unbounded increase in complexity. During open-ended evolution, measures for evolution, such as the number of possible **genotypes**, increase while the realized **phenotypes** increase more slowly or even decrease. As a result, the fraction of realized versus possible **phenotypes** steadily decreases during open-ended evolution. Evolutionary measures are the increase or decrease in number, diversity, novelty, and complexity of genotype and phenotype over time. This process can be subdivided into weak, strong, and ultimate.[7]*

***Compartments** are a spatial organization of chemical systems, like droplets and vesicles, that prevent homogenization within their environments. Compartments also offer protection from the surrounding environment.[6]*

***Cells** are the basic structural and functional unit of life forms. The term was established by Hooke long before molecular basis was known. Therefore, we propose to generalize the term synthetic cell to include systems that use non-biological building blocks based on assemblies other than vesicles, e.g., droplets.*

***Synthetic Life.** An umbrella term encompassing the terms Synthetic Cell and de Novo Life. We defined synthetic life as a self-sustaining chemical system from non-living matter capable of open-ended evolution.*

> ***De novo life.** The systems chemistry community favors this term and means life from synthetic building blocks.*
>
> ***Synthetic cell.** The minimal living entity of synthetic life. The terms synthetic cell, artificial cell, or minimal cell are often used as synonyms.*
>
> ***Artificial cell:** Predominantly used to describe cells that contain not only biological building blocks.*
>
> ***Minimal cell:** A synthetic cell that is constructed (from the bottom up or top down) to identify minimal sets of components for a given function.*
>
> ***Protocell**: A protocell is a precursor of a cell, which is engineered using components that may have been present at the origins of life in the transition phase between chemical and biological evolution. The term protocell is, therefore, predominantly used by the origins of life community.*

Box 2 List of hallmarks

Hallmarks that are fundamental prerequisites of life

Self-sustaining

Self-replicating

(Randomly) Mutating

Open-endedly improving through a selection of the fittest

Hallmarks Associated with Life

Compartment

Growth and development

Metabolism

Reproduction

Responding to stimuli

Adaptation through evolution

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Author Contributions

All listed authors have participated in the discussions that led to this manuscript in the context of the Engineering Life workshop held from 13.04.2023 – 24.03.2023 in the MIAPbP center at Boltzmannstr. 2, 85748 Garching. J.B., K.G., C.A.W., and C.M.E.K. have written the manuscript. The following authors were invited to the workshop and gave a keynote lecture and a discussion on a selected topic that was key to the manuscript: J.B., K.G., C.A.W., K.A., E.S.A., C.B., D.B., E.F., U.G., W.T.S.H., F.J., N.L., L.M., S.O., J.S., P.S.

During our workshop, we experienced that reaching a consensus with an interdisciplinary crowd is challenging. Indeed, not every author agrees on every point discussed in this perspective, which lies in the inherent nature of the topic and such an article. Nevertheless, all authors have agreed to publish this perspective as a valuable resource to the field.

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