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Perspective

A roadmap toward the synthesis of life

Christine M.E. Kriebisch,[1] Olga Bantysh,[2] Lorena Baranda Pellejero,[3] Andrea Belluati,[4,5] Eva Bertosin,[6] Kun Dai,[7] Maria de Roy,[3] Hailin Fu,[8] Nicola Galvanetto,[9] Julianne M. Gibbs,[10] Samuel Santhosh Gomez,[11] Gaetano Granatelli,[11] Alessandra Griffo,[12,13] Maria Guix,[14] Cenk Onur Gurdap,[15] Johannes Harth-Kitzerow,[1,16,17,28] Ivar S. Haugerud,[11] Gregor Häfner,[18,19] Pranay Jaiswal,[11] Sadaf Javed,[20] Ashkan Karimi,[1] Shuzo Kato,[21] Brigitte A.K. Kriebisch,[1] Sudarshana Laha,[22,35] Pao-Wan Lee,[23] Wojciech P. Lipinski,[20] Thomas Matreux,[24] Thomas C.T. Michaels,[25,26]

(Author list continued on next page)

[1]School of Natural Sciences, Technical University of Munich, 85748 Garching, Germany
[2]Department of Materials Science and Physical Chemistry and Institute of Nanoscience and Nanotechnology (IN2UB), University of Barcelona, 08028 Barcelona, Catalonia, Spain
[3]Life-Like Materials and Systems, Department of Chemistry, University of Mainz, Düsbergweg 10-14, 55128 Mainz, Germany
[4]Department of Pure and Applied Chemistry, University of Strathclyde, Thomas Graham Building, 295 Cathedral Street, Glasgow G1 1XL, UK
[5]Department of Chemistry and Centre for Synthetic Biology, Technical University of Darmstadt, Peter-Grünberg-Straße 4, 64287 Darmstadt, Germany
[6]Department of Bionanoscience, Kavli Institute of Nanoscience, Delft University of Technology, Delft 2629 HZ, the Netherlands
[7]University of Freiburg, Cluster of Excellence livMatS, FIT–Freiburg Center for Interactive Materials and Bioinspired Technologies, Georges-Köhler-Allee 105, 79110 Freiburg, Germany

(Affiliations continued on next page)

THE BIGGER PICTURE Challenges and opportunities:

- Synthetic life deepens our understanding of the fundamental principles of life and can help unveil mechanisms that operated at life's emergence. But it is more than just an academic pursuit—it opens the door to creating novel life forms with practical applications. From biotechnology and medicine to materials science, synthetic life has the potential to revolutionize industries and deliver tangible societal benefits.
- Social, philosophical, and technical challenges, such as the lack of a broadly accepted definition, vague goals, misaligned interdisciplinary efforts, and public and ethical concerns, hinder the progress of synthesizing life.
- This perspective offers a cross-disciplinary roadmap toward synthetic life that does not shy away from the challenges, ethical concerns, and provocative implications. Compiled over a 2-week workshop involving 57 scientists from 14 countries, we highlight technical and non-technical challenges for the scientific community to embrace a bold, unified vision for the future of synthetic life.

SUMMARY

The synthesis of life from non-living matter has captivated and divided scientists for centuries. This bold goal aims at unraveling the fundamental principles of life and leveraging its unique features, such as its resilience, sustainability, and ability to evolve. Synthetic life represents more than an academic milestone—it has the potential to revolutionize biotechnology, medicine, and materials science. Although the fields of synthetic biology, systems chemistry, and biophysics have made great strides toward synthetic life, progress has been hindered by social, philosophical, and technical challenges, such as vague goals, misaligned interdisciplinary efforts, and incompletely addressing public and ethical concerns. Our perspective offers a roadmap toward the synthesis of life based on discussions during a 2-week workshop with scientists from around the globe.

INTRODUCTION AND MOTIVATION

Synthesizing life from non-living matter 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. Over the past decades, efforts in synthetic biology, systems chemistry, and beyond[1–7] have resulted in great progress toward this goal. For example,

Erik Poppleton,[12,13] Alexander Ruf,[27,28] Annemiek D. Slootbeek,[20] Iris B.A. Smokers,[20] Héctor Soria-Carrera,[1] Alessandro Sorrenti,[29] Michele Stasi,[1] Alisdair Stevenson,[25,26] Advait Thatte,[11] Mai Tran,[12,13] Merlijn H.I. van Haren,[20] Hidde D. Vuijk,[11] Shelley F.J. Wickham,[30] Pablo Zambrano,[1] Katarzyna P. Adamala,[31] Karen Alim,[1,19,32] Ebbe Sloth Andersen,[33] Claudia Bonfio,[34] Dieter Braun,[19,24] Erwin Frey,[19,35] Ulrich Gerland,[1] Wilhelm T.S. Huck,[20] Frank Jülicher,[19,22,36,37] Nadanai Laohakunakorn,[38] L. Mahadavan,[39] Sijbren Otto,[40] James Saenz,[41] Petra Schwille,[19,42] Kerstin Göpfrich,[12,13,19,*] Christoph A. Weber,[11,*] and Job Boekhoven[1,19,*]

[8]Institute for Complex Molecular Systems and Laboratory of Macromolecular and Organic Chemistry, Eindhoven University of Technology, P.O. Box 513, Eindhoven 5600 MB, the Netherlands
[9]University of Zurich, Winterthurerstrasse 190, Y-44-K-96, 8057 Zürich, Switzerland
[10]Department of Chemistry, University of Alberta, Edmonton, AB T6G 2G2, Canada
[11]Faculty of Mathematics, Natural Sciences, and Materials Engineering, Institute of Physics, University of Augsburg, Universitätstrasse 1, 86159 Augsburg, Germany
[12]Biophysical Engineering Group, Max Planck Institute for Medical Research, Heidelberg, Germany
[13]ZMBH, Center for Molecular Biology, Heidelberg University, Heidelberg, Germany
[14]Department of Materials Science and Physical Chemistry and Institute of Theoretical and Computational Chemistry, University of Barcelona, 08028 Barcelona, Catalonia, Spain
[15]Science of Laboratory, Department of Women's and Children's Health, Karolinska Institute, 17165 Solna, Sweden
[16]Department of Physics, Ludwig Maximilians Universität, Munich, Germany
[17]Max Planck Institute for Astrophysics, Karl-Schwarzschild-Str. 1, 85741 Garching, Germany
[18]Institut für Theoretische Physik, Georg-August Universität Göttingen, Friedrich-Hund Platz 1, 37077 Göttingen, Germany
[19]Max Planck School Matter to Life, Heidelberg, Germany
[20]Institute for Molecules and Materials, Radboud University, Heyendaalseweg 135, Nijmegen 6525 AJ, the Netherlands
[21]Department of Physics, Kyushu University, Motooka 744, Fukuoka 819-0395, Japan
[22]Max Planck Institute for the Physics of Complex Systems, Nöthnitzer Strasse 38, 01187 Dresden, Germany
[23]Institute of Bioengineering, School of Engineering, École Polytechnique Fédérale de Lausanne (EPFL), Station 17, 1015 Lausanne, Switzerland
[24]Systems Biophysics Center for Nanoscience and Origins Cluster Initiative, Department of Physics, Ludwig-Maximilians-Universität Munich, Geschwister-Scholl-Platz 1, 80539 München, Germany
[25]Department of Biology, Institute of Biochemistry, ETH Zurich, 8093 Zurich, Switzerland
[26]Bringing Materials to Life Initiative, ETH Zürich, Zurich, Switzerland
[27]LMU Munich, Faculty of Physics, Schellingstraße 4, 80799 Munich, Germany
[28]Excellence Cluster ORIGINS, Boltzmannstraße 2, 85748 Garching, Germany
[29]Department of Inorganic and Organic Chemistry (Section of Organic Chemistry), University of Barcelona (UB), 08028 Barcelona, Spain
[30]School of Chemistry, The University of Sydney, Sydney, NSW 2006, Australia
[31]Department of Genetics, Cell Biology and Development, University of Minnesota, Minneapolis, MN, USA
[32]Max Planck Institute for Dynamics and Self-Organization, Göttingen, Germany
[33]Interdisciplinary Nanoscience Center (iNANO) and Department of Molecular Biology and Genetics, Aarhus University, 8000 Aarhus, Denmark
[34]Institute of Science and Supramolecular Engineering (ISIS), CNRS UMR7006, University of Strasbourg, Strasbourg, France
[35]Arnold Sommerfeld Center for Theoretical Physics and Center for NanoScience, Ludwig-Maximilians-Universität München, Munich, Germany
[36]Center for Systems Biology Dresden, Pfotenhauerstrasse 108, 01307 Dresden, Germany
[37]Cluster of Excellence Physics of Life, TU Dresden, 01062 Dresden, Germany
[38]Institute of Quantitative Biology, Biochemistry, and Biotechnology, School of Biological Sciences, University of Edinburgh, Edinburgh, UK
[39]School of Engineering and Applied Sciences, Department of Physics, Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA, USA
[40]Centre for Systems Chemistry, Stratingh Institute, University of Groningen, Nijenborgh 3, Groningen 9747 AG, the Netherlands
[41]TU Dresden, B CUBE Center for Molecular Bioengineering, Tatzberg 41, 01307 Dresden, Germany
[42]Max Planck Institute of Biochemistry, Martinsried, Germany
*Correspondence: k.goepfrich@zmbh.uni-heidelberg.de (K.G.), christoph.weber@physik.uni-augsburg.de (C.A.W.), job.boekhoven@tum.de (J.B.)
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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[9,10] and DNA or RNA nanotechnology.[11,12] Furthermore, systems chemistry, which explores how interacting chemical reaction networks can give rise to emergent properties, uses entirely non-biological molecules for engineering synthetic cells, complementing the traditional biological approach.[6,13–41] Excitingly, some works show elements of Darwinian evolution, defying the long-standing belief that Darwinian evolution is a biological phenomenon.[6,36–40] Yet, significant technical and conceptual challenges remain. One major challenge to the synthesis of life is the lack of clarity on the goals. Moreover, communication around synthetic life is no longer a conversation among scientists but involves the public, sensitive to misunderstanding and fear, easily amplified by sensationalized media narratives.

Despite the challenges, synthetic life has far-reaching potential in academia and industry. We aim to learn about what life is, its

Box 1. List of criteria for life

Criteria 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.

minimum requirements, and what would be needed for its existence in niches beyond Earth or at the origin of life—fields of research that strongly overlap. Beyond these fundamental questions, the pursuit of synthetic life is increasingly driven by its practical potential in biotechnology, medicine, and materials science. For example, synthetic cells are not only exciting academic tools but also have the potential to become transformative biofactories, producing high-value pharmaceuticals, breaking down persistent pollutants, capturing greenhouse gases, and redefining energy storage systems.[4,42,43] Directed evolution, groundbreaking for developing new enzymes, gives a first glimpse into the opportunities for the evolution of synthetic cells. Evolving synthetic cells will further accelerate the rapid growth of the global SynBio market.[44] Because they can be evolved to perform one specific task, they can surpass the efficiencies of natural cells. 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. What makes this endeavor even more compelling are the potential technological breakthroughs along the way. From advanced gene assembly methods, such as Gibson assembly,[45,46] to high-throughput selection techniques and information encoding into non-biological heteropolymers, the synthesis of life is not just an end goal—it is a driving force for technological innovation across disciplines.

In this perspective, we offer a roadmap toward synthetic life that does not shy away from the challenges, ethical concerns, and provocative implications. Compiled over a 2-week workshop involving 57 scientists from 14 countries, we highlight technical and non-technical challenges for the scientific community and put forward a bold, unified vision for the future of synthetic life.

DISCUSSION

What is the overarching goal?

Engineering synthetic life requires clear, actionable targets and milestones to measure the progress of the field. Central to this challenge is the need for a definition of life. Although many have been proposed,[47,48] none have achieved a broad acceptance. Instead of defining life, others have taken a less controversial approach by listing a set of hallmarks, such as metabolism, compartmentalization, replication, motility, and response to stimuli, and trying to incorporate these into non-living systems. We advocate against this approach because these definitions tempt us to realize one hallmark after another and leave their integration until the end (see, for example, Box 1). The strategy leads to exciting systems with "life-like" traits and important insights into the workings of biology.[43,49] However, this approach is unlikely to 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, to synthesize life, we should aim for chemical systems that can undergo open-ended evolution. In such a system, we expect life-like features like those listed above to emerge naturally through evolution.[48] Put differently, goals like responsiveness or motility should no longer be treated as endpoints. They are not the essence of life but byproducts of evolution. To synthesize life, we must shift our ambitions away from achieving individual traits and toward creating systems that can evolve unpredictably and endlessly.

Thus, we 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 (Figure 1A).[6,7,50–52] A *chemical system* implies that we are dealing with molecules in a chemical reaction network, which sets this field apart from *artificial life*. Life is *self-sustaining*—it continues to operate with precursors, building blocks, and energy offered by the environment. *Metabolism*, both anabolism and catabolism, is responsible for self-sustainment: environmental precursor molecules are converted into the building blocks for life. Moreover, synthetic life should be synthesized from non-living matter. That means that the building blocks for the self-sustaining system cannot be based on living systems. However, synthetic life can be synthesized from biologically derived molecules like DNA, reconstituted or purified proteins, or even dead cells.

Synthetic life must evolve following the principles of biological *Darwinian or Lamarckian evolution*. To do so, it must be able to replicate—autonomously copy 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 that all other critical components, such as a new compartment and catalysts for metabolism, are also self-synthetized. Note that information can refer to a specific sequence of a polymer or the molecular configuration of molecules. 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 (Figure 1B). Under those conditions, one can expect a never-ending evolution yielding surprising solutions, including the list of hallmarks mentioned above (see Box 1).

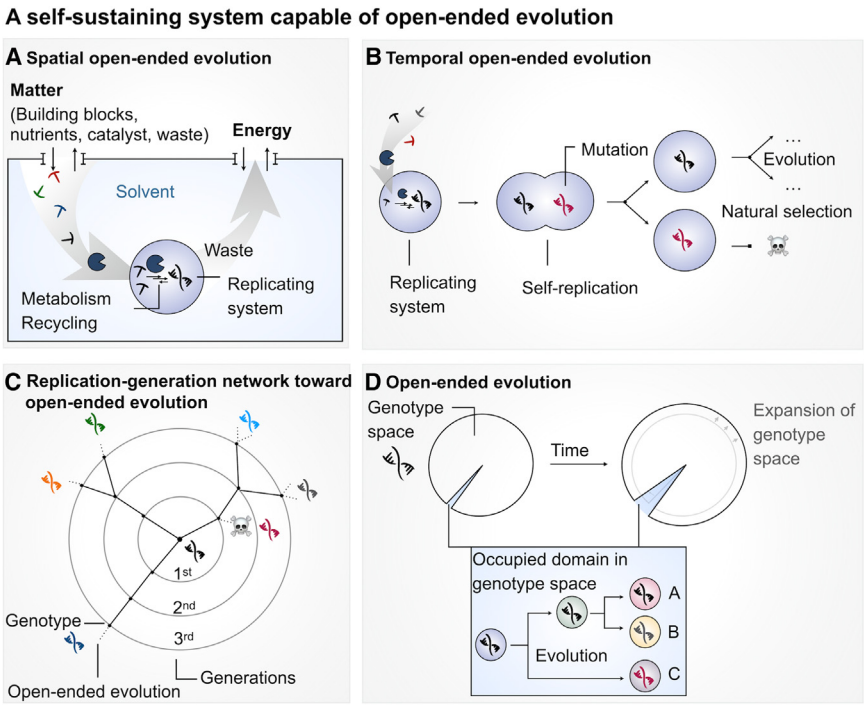

Figure 1. The overarching goal: Synthetic life from non-living building blocks

(A and 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, whereas the weaker ones decay.

(C and 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.

The breadth of the goal means life can be synthesized using a broad choice of building blocks—from biological hardware to entirely synthetic molecules and everything in between. This diversity of choice is an advantage and simultaneously challenges conventional thinking that life should be made of biological molecules. Moreover, the selection of building blocks fundamentally shapes the research questions. Synthetic life from simple, prebiotically plausible molecules can unveil mechanisms that operate at the origin of life. On the other hand, synthetic life, designed with highly evolved biomolecular machinery, has the power to outperform natural systems.

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 living.[3] Instead, we aim for a living version of a synthetic cell, i.e., a "living synthetic cell." The systems chemistry community does not 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. "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 2).

The state of the art

Significant progress has been made across disciplines like systems chemistry, biophysics, synthetic biology, and DNA/RNA nanotechnology, each contributing to the synthesis of life through distinct molecular building blocks and environments.[1–7,10,15–19,41,43,55] Here, we highlight critical developments, moving from natural building blocks to increasingly synthetic hardware (Figure 2), rather than providing an exhaustive review of the literature.

The most obvious way to synthesize life may be to start with a pre-existing 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 has the ability to reproduce.[56] Today's version of this cell, JCVI-syn3.0, has as little as 473 genes[57] some of which are involved in crucial processes, such as transcription and translation, whereas others have unknown but critical functions.[58] 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[1,59] containing all components to start the transcription and translation processes.[1,4,43,60,61]

Bottom-up synthetic biology aims at yet simpler versions of synthetic cells 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.[1,4,43] In this way, specific hallmarks of life, such as energy conversion,[62,63] 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.[1,8,64–67] However, it remains a great challenge to self-replicate all necessary components.[68] 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.[66] 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, lipids,

Box 2. Glossary

A **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 itself. 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.[53]

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

A self-sustaining chemical system is a chemical system that can regenerate all of its system's components and without intervention by a higher entity, such as us scientists.[50]

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.[36]

In biology, the **genotype** of an organism is defined as its complete set of genetic material, i.e., an essential fraction of 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, in particular, emergent 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, an entity gets modified during its "lifetime" and passes this modification on to its offspring.

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, the 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 define 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, which means life from synthetic building blocks.

Synthetic cell. The minimal compartment of synthetic life. The terms synthetic cell, artificial cell, or minimal cell are often used as synonyms.

Artificial cell. A minimal compartment 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 made using components that 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.

and proteins to accomplish division and regrowth cycles based on fewer, simpler components. For instance, the genetic encoding of the production of compartment-forming peptides was demonstrated.[69] Still, in such an approach, many components must transcribe and translate these peptides, 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,[70] cytoskeletal filaments,[71] or compartments.[72,73] 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.[74,75] Recent progress on the co-transcriptional folding of RNA origami[76,77] 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 for compartment formation[13–33,41] and chemical systems capable of replicating themselves without using complex biological machinery.[52,78] For example, DNA has been demonstrated to replicate using

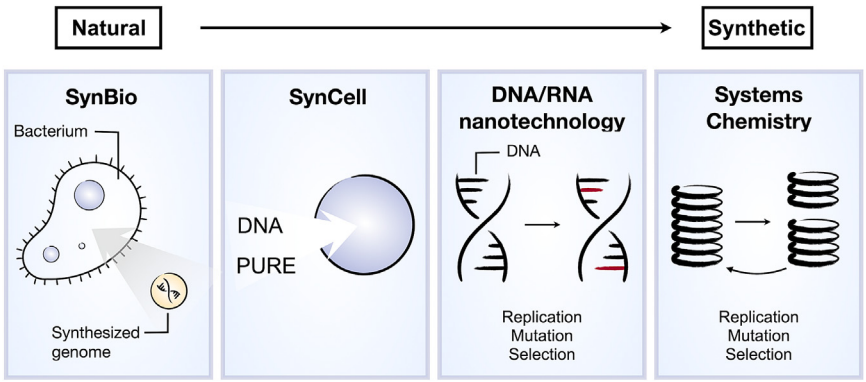

Figure 2. The current state of the art in synthetic life

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).

non-natural, chemically activated nucleotides.[79–81] DNA origami has yielded insights into encoding and copying structures where information is not directly encoded in the DNA bases.[82] Ideas have been put forward on the self-replication and evolution of DNA crystals.[83] Besides DNA, self-replicators based on RNA,[84,85] peptides,[86,87] and non-biological building blocks exist.[54,78,88–95] 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.[96] Moreover, self-replicating molecules can catalyze reactions besides replication, which allows for metabolic reaction networks needed for open-ended evolution.[52] Recent work has shown that self-replicating stacks of macrocycles can catalyze other chemical reactions besides their formation.[37,97] Combined with years of work on using chemical reaction networks to regulate molecular self-assembly,[24,38,49,88,98–109] it opens the door to a catalytically active genotype that also regulates its environment.[110]

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

Synthetic life attracts scientists from classical biology, systems chemistry, DNA/RNA nanotechnology, biophysics, and more. Yet, despite decades of research, we still need to work on cohesive terminology. Effective communication requires a commonly understood language. Biologists have studied life for centuries and meticulously developed a language to describe life's intricacies. Historically, these terms were only relevant to life as we know it. Thus, terms like Darwinian evolution, genotype, and phenotype are tightly related to a limited set of biomolecules. As discussed, synthetic life may use different molecules, 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. Synthetic life must be described with biological terms, provided 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. We should highlight the generalization whenever we use a biological term to describe a system not based on biological building blocks. Similarly, we must remain self-critical and highlight the limitations of the analogies we draw. We need to ensure that the terms first coined by biologists are not used to oversell results or in ways that are 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 2. 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. The synthetic life community must regard communication as a central, essential effort to achieve maximal transparency and, ultimately, the acceptance of a new manufacturing paradigm.

Conventional measures of science communication often only reach groups with a high level of prior information. Therefore, a synthetic life communication strategy has to be crafted to reach out to harder-to-reach target groups. 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 societies' 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 supporting open-ended evolution must be chosen—one that does it all. That means all chosen building blocks must act interoperably to achieve the minimum requirements for

life. This approach may contrast with the classical approach of designing separate life-like features into different non-living systems. However, the field does not need to converge on a single molecular framework; diverse chemical systems, from nature-inspired to entirely synthetic, can lead to multiple forms of synthetic life (Figure 2). A multidisciplinary approach 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. Selecting the right environment and energy source is critical—whether simple molecules, enzymes, or light and temperature gradients. 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 surroundings. If the energy conversion rate is low, replication is slow, and related non-equilibrium phenomena such as force generation are negligible.[111] 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.[112] Parasitic behavior, in which one self-replicating system depends on the building block of another, has also been explored.[113]

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.[113] A challenge with such alternative decay mechanisms is that they select for the fastest replicator, colloquially referred to as Spiegelman's monster.[114] Creative methods involve compartmentalization to prevent a takeover by the faster replicating molecular parasites,[115] 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 2). We can challenge the necessity of genotype-phenotype coupling through the transcription-translation machinery for synthetic life. Although 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 that 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 also be designed for synthetic life.[116] For example, the information-encoding molecules could perform functions besides carrying information. Self-replicating RNA systems with limited complexity function as the genetic material, structural component, and catalyst,[117] which 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, this concept applies to completely non-biomolecular self-replicating systems, such as the self-replicating molecular stacks of macrocycles[37,97] described in the state of the art.

Even when genotype and phenotype are coupled, challenges exist. Although 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*.[118,119] Suitable genotype-phenotype maps have to fulfill a set of properties that are essential for their evolvability[119]: (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, whereas others are encoded only by a few. (3) Robustness: a certain fraction of possible mutations leaves 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.

Noteworthy, redundancy (multiple genotypes mapping to the same phenotype) does imply robustness (some mutations not

changing the phenotype). However, they are often considered distinct because redundancy focuses on the genetic diversity leading to the same outcome, whereas robustness emphasizes the system's resilience to changes. It is like having multiple routes to the same destination (redundancy) versus being able to handle roadblocks without losing your way (robustness).

Tuning mutation rates

Darwinian evolution is impossible without mutations in the genotype, which allow 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.[120] 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[121] 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 sequence space. For DNA/RNA-encoded synthetic life, methods developed in directed evolution can be used to fine-tune mutation rates by designing appropriate DNA libraries. For the system to autonomously tune mutation rates, it is possible to use DNA polymerases with appropriate error rates, like the Taq polymerase[122] or use other physical and chemical factors.[123–125] 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 2). 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 (Figure 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. Although established theories have been proposed on the requirements and measures for open-ended evolution,[126,127] it is a significant challenge to implement those in synthetic systems. As such theories are based on general principles, open-ended evolution should be realizable with different sets of molecular hardware, which allows for diverse approaches toward 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.

The first approach involves scoring systems based on the fundamental prerequisites of life. For example, a vesicle with a self-replicating genotype is further from life than one with a self-replicating and mutating genotype. These prerequisites can be quantified further, such as through replication rates or fidelity. Consequently, a non-zero value for this score does not imply a system is living, and there is no threshold for transitioning from a living to a non-living system.

The second approach uses 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.[119] A recent framework, the assembly theory, uses the number of molecule copies and the assembly index to describe how novelty is generated. The assembly index assesses the minimal number of recursive steps required for building the molecule. The number of copies is essential to discriminate from randomness, whereas the assembly index assesses the complexity of a molecule.[128]

Establishing ethical considerations

Ethical considerations regarding the risks, accountability, responsibility, value of life, and public perception must be continuously re-evaluated when synthesizing life. The possibility of synthetic life escaping containment and wreaking havoc on natural ecosystems is not hypothetical. Authorities realize that we need safety procedures, like those stated in biological sciences, to prevent the escape of genetically modified organisms for synthetic life (e.g., dedicated synthetic life labs with containment procedures).[129] Ideally, safety procedures are controlled by international organizations like iGem for synthetic cell research, which has already been demonstrated (https://responsibility.igem.org/). Moreover, history has shown us the duality of scientific advancements. For example, organic chemistry brought life-saving medicines and chemical weapons. Synthetic life is no different, and without the immediate implementation of biosecurity measures, the potential for misuse, whether accidental or malicious, is vast and terrifying.

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. Noteworthy, synthetic life and artificial intelligence communities share some ethical considerations, including moral responsibility, safety and risks, transparency, and accountability.[130] As synthetic life relies on chemical entities, while artificial intelligence does not, both communities have different risk mitigation strategies and must follow different regulations.

CONCLUSIONS

The synthesis of life is a fascinating scientific endeavor and challenges our understanding of life in the natural world. This perspective offers a roadmap for realizing synthetic life by addressing ten critical technical and non-technical challenges. 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—both within the community and 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 contribute to establishing such a language by defining terms in the Glossary in Box 2. 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 the synthesis of life is achievable in the coming decades. However, there are massive challenges ahead and more to come.

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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 2023-3-13 to 2023-3-24 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 held 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., and 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 is inherent to the nature of the topic and such an article. Nevertheless, all authors have agreed to publish this perspective as a valuable resource to the field.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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