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A Primer on Building Life-Like Systems

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The quest to understand life and recreate it in vitro has been undertaken through many different routes. These different approaches for experimental investigation of life aim to piece together the puzzle either by tracing life's origin or by synthesizing life-like systems from non-living components. Unlike efforts to define life, these experimental inquiries aim to recapture specific features of living cells, such as reproduction, self-organization or metabolic functions that operate far from thermodynamic equilibrium. As such, these efforts have generated significant insights that shed light on crucial aspects of biological functions. For observers outside these specific research fields, it sometimes remains puzzling what properties an artificial system would need to have in order to be recognized as most similar to life. In this Perspective, we discuss properties whose realization would, in our view, allow the best possible experimental emulation of a minimal form of biological life.

1. Introduction

Life, as we know it, is a puzzle that has enthralled generations of researchers. As a phenomenon, life is difficult to define,[1,2] is multifaceted, and thwarts any attempt to understand it from a singular perspective. Since a complete understanding of life entails the ability to synthesize living entities, experimental investigations have been directed towards the synthesis of cells with life-like properties from their non-living components. This involves understanding either the emergence of life under credible prebiotic conditions, or building synthetic cells from 'parts' sourced from extant biology.[3] Either approach involves recapturing specific properties of living organisms, the underlying chemistry and biophysical constraints. In the experimental investigations aimed to transition non-living matter into living organisms, however, the properties of living organisms that are targeted are not subject to consensus.[4]

A useful metaphor to understand synthetic life research is the parable of the blind men appraising an elephant – a creature they have never encountered before (Figure 1). Each blind man touches a particular part of the elephant and announces his opinion accordingly. So, the blind man who touches the elephant's trunk declares that the elephant is like a giant snake, whereas the one who feels it legs announces it to be like a tree. The parable is often evoked to convey limitations of individual perception, which may be true, but may not constitute the whole truth. Our attempts to understand this "beast" called life are similar, and although these attempts do not explain the phenomenon of life in its entirety, they do shed light on particular aspects of it. In this Perspective, we seek to highlight such specific aspects of life for people outside the synthetic cell/origins of life community.

Figure 1. Blind monks examining an elephant", an ukiyo-e print by Hanabusa Itchō, Public domain, via Wikimedia Commons.

The emergence of molecular building blocks of life in putative prebiotic environments has been extensively studied in the field of prebiotic chemistry.[5] Numerous credible pathways towards the synthesis of biomolecules, such as nucleotides,[6] peptides[7] and metabolites,[8] have been proposed. However, the question remains as to how these building blocks assembled into early living systems and whether this can be recreated experimentally. A similar same problem arises in case of modern biological macromolecules: They are accessible from various biological sources yet it is unclear if they can be used to (re-)create living, cell-like structures from scratch.

Our goal here is to provide an overview of the properties that we believe should be the focus of experimental investigations into the emergence and/or synthesis of life-like artificial systems. We list the following four characteristics as hallmarks of living systems: Self-replication, evolution, robustness and autonomy (Figure 2). This is not an exhaustive list of

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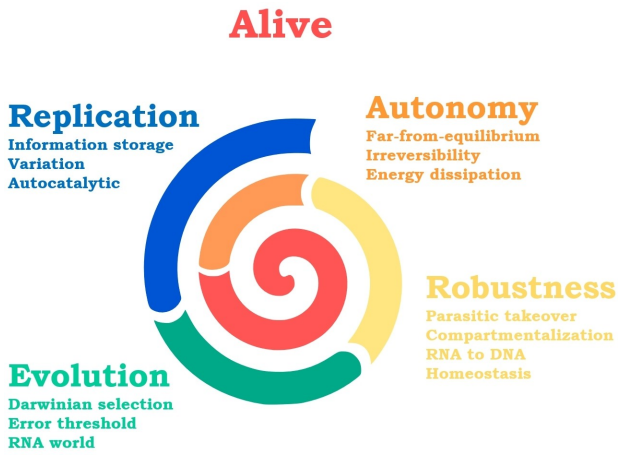

Figure 2. The four aspects of living organisms targeted in the experimental study of life.

all attributes of living beings, but is meant to provide a taste of nuances involved in the study of artificial life-like systems.

2. Self-Replication

Self-replication can be observed at all levels of biological systems, from simple viroids, viruses, and protozoa to multicellular organisms and their collectives. Life propagates by reproducing itself. From a reductionist perspective, self-replication can be traced down to the molecular level – the replication of nucleic acids (genotypes) which encode the inheritable traits and functions (phenotypes). Although replication is a property observed at all levels of cellular self-organization including membrane compartments, which can, to some extent be recaptured using cytomimetic structures, such as micelles,[9] liposomes[10] and nanostructures,[11] we limit our focus here to the replication of genetic polymers, the informational subsystems that lie at the core of biological self-replication.[12]

Template-dependent replication of genetic information, particularly catalyzed by proteins, is the primary mechanism of information storage and inheritance in biological genetic systems. Even in case of origins of life studies, non-enzymatic replication of nucleic acids is considered as the basis of progression in the development of living systems.[13–16] For example, the RNA world hypothesis postulates that the emergence of autocatalytic, self-replicating RNA molecules marked a major evolutionary transition, wherein all life forms were once entirely based on RNA.[17–19]

Since the replicating unit itself catalyzes its own formation, the self-replication process is autocatalytic. The rate of the replication reaction becomes a power function of the concentration of the replicating unit.[20] In case of a replicating species R, the rate of the replication reaction is expressed as, $d[R]/dt = k \cdot [R]^n$, where $[R]$ is the concentration, k is the rate constant and n is the order of reaction.[9] The value of n determines the 'strength' of autocatalysis. Values of $n = 0.5$, 1 and 2 result in parabolic, exponential and hyperbolic growth functions, respectively. Templated self-replication is ideally exponential, i.e., $n = 1$. However, product inhibition, such as by association of template strands to form dimers, leads to sub-exponential – parabolic growth $(n = 0.5)$, as was observed in the case of non-enzymatic templated self-replication by condensation of trinucleotides.[13] In order to exhibit Darwinian behavior (selection of fittest species, i.e., species with highest k),[21] exponential autocatalytic self-replication is thought to be essential.[9,22] This is not the case for parabolic growth, which, in case of competition, leads to stable coexistence of all species, i.e. "survival of everybody".[21,22] Autocatalytic self-replication of informational polymers was experimentally achieved, for example, for immobilized DNA oligonucleotides[23] and peptide-based[24] replicators as well as cross-catalytic ligase ribozymes,[25] Much more complex self-replicating systems based on in vitro translation coupled DNA and RNA replication by polymerase enzymes were also described.[26–28] However, these systems are not genuine autocatalytic replicators in the strict sense, as they require the presence of a complex translational machinery that is not part of the replicative cycle (more on this in the "autonomy" section). Nevertheless, these systems are important contributors to the generation of semi-autonomous and evolvable life-like systems. Generally, experimental examples are not frequent and developing self-sustained genetic replicators capable of exponential growth remains challenging.

Although the aspect of self-replication is, in our opinion, one of the most important in terms of establishing a life-like system, it needs to be combined with variation and selection to lead to mechanisms for increasing genetic information and complexity. This leads us to the fundamental property exhibited by all extant life: the ability to evolve.

3. Evolution

Closely related to self-replication is the ability of known organisms to undergo heritable evolutionary adaptation. As such, evolution is part of many definitions of the phenomenon life including the popular "NASA working definition": "Life is a self-sustaining chemical system capable of Darwinian evolution".[18,29] Although this definition also suffers from contradicting example (infertile organisms like mules),[2,30] it is quite broad and mature, as all living systems on this planet are products of evolutionary processes.

At least a minimal level of inheritable adaptation can therefore be considered as one of the most important experimental criteria for the generation of life-like systems. Darwinian evolution at the most basic level involves error-prone (self-)replication of variants with inheritable information, followed by environmental selection of 'fit' variants. Darwinian evolution thus consists of three processes: replication, mutation and selection. Moreover, the evolutionary process is open-ended,[31] i.e. the complexity and diversity of offspring can increase over successive generations indefinitely in an open-ended manner. As a first attempt to understand the origin of life in the context of extant biology progressed, a physicochem-

ical and molecular basis of evolution was sought. Sol Spiegelman's landmark experiments in 1967 implemented natural selection in vitro using Qβ RNA molecules, thereby initiating a new field of inquiry: in vitro selection.[32] Pioneering experiments in in vitro selection provided numerous insights into the dynamics of evolutionary processes.[16,33] These studies showed that 'inanimate' molecules were capable of exhibiting behavior analogous to living organisms subject to natural selection. In other words, the chemical and biological domain no longer seemed so separate. While the theory of evolution (in particular in its extended variants[34]) provides a coherent explanation of the diversification and proliferation of life over the course of the last 4 billion years, it does not explain the rise of chemical systems capable of evolution itself. Following the aforementioned NASA definition of life, the rise of molecular systems capable of Darwinian evolution would then be considered as the origin of life itself.[35] Indeed, this is what the RNA world hypothesis and related hypotheses postulate: That life arose as a set of genetic polymers capable of autocatalytic replication, with the polymers serving as both genotype (encoded sequential information) and phenotype (structure and associated properties that determine 'fitness' of variants).[17,36]

An implicit requirement for evolution is the ability to accommodate variation over successive generations, with mutations being the source of variation. Given that the evolutionary process necessitates variation by mutation, the rate of mutation determines the rate of evolutionary progression.[35] This, however, creates a problem in terms of effective information storage across successive generations. Consider for example, Spiegelman's in vitro selection experiment,[32] which was initiated using the RNA genome of bacteriophage Qβ. This RNA genome encodes only 4 genes, namely the coat protein, maturation protein, replicase subunit and lysis protein. As the replication and selection proceeded, successive generations of RNA strands became shorter, to the point where they lost all protein encoding genes and were left only with the recognition motifs necessary for recognition and replication by the replicase. Thus, in the absence of high-fidelity replication or strong selection pressure, mutations are rife and can lead to deterioration of information content across successive generations. Low fidelity and processivity presumably also limited the length of the replicating sequence in early biological systems. This problem was elaborated in the form of "Eigen's paradox", which can be well illustrated for RNA replication by ribozymes. Joyce and co-workers used innovative in vitro evolution protocols to improve an RNA polymerase ribozyme.[37] One of the selected ribozymes could catalyze single-turnover copying of functional RNA sequences with substantial secondary structure, such as aptamers and ribozymes. Yet multi-turnover amplification of sequence information was limited to small templates of 20–25 nt, owing to limited fidelity and inefficient strand displacement of the polymerase. Thus, sustained replication of long nucleic acid genomes requires either modern polymerases enzymes or hypothetical, much more complex ribozymes e.g. with helicase and proof-reading domains. Paradoxically, the evolution of such complex catalysts requires a priori replication of even longer nucleotide sequences, which is seemingly unattainable for more primitive precursor enzymes. Eigen resolved this catch-22 by postulating the hypercycle principle.[14] The hypercycle is a closed network of self-replicating molecules which catalyze each other's production. Even if the amount of information (sequence length) of each member of the hypercycle is limited, collectively the members can overcome the error threshold. The hypercycle principle thus raised the possibility of increasing the information content, leading to evolution of increasingly complex living systems. However, a lethal flaw to this concept was pointed out by Maynard-Smith,[38] in that the hypercycle is vulnerable against parasites: Rogue replicators, that do not encode meaningful (genotypic/genetic) information, are shorter and as a result, replicate rapidly. Although ligation-based replication mechanisms and length-selective environments can suppress parasitization and defect catastrophizing to some extent, the evolutionary possibility of such systems may be limited.[39] Life, however, has managed to suppress parasitic growth and increased the phenotypic complexity over time. It has been able to effectively transfer increasingly complex inheritable information from genotype to phenotype without being overwhelmed by parasitic growth, in the face of ever-changing environmental perturbations. In other words, life is robust. This brings us to the third aspect that is common to living organisms and should therefore also be a key aspect of experimental systems aiming at creating synthetic life-like systems: robustness.

4. Robustness

The concept of robustness, like the concept of life, is used in a broad range of contexts[40] and as a result loses clarity. Robustness implies stability in the face of perturbations. However, it is also important to describe both the nature of the stability as well as the perturbations.[41] In other words, the robustness of a system can be described only by describing what aspect of the system is robust against what kind of perturbations. In the case of biological systems, robustness can be defined as resilience against detrimental genetic and environmental factors. Genetic robustness may, for example, refer to robustness to takeover by parasitic sequences[35] or chemical damage, as reflected, for example, in the higher stability of DNA compared to RNA.[42] On the other hand, robustness with respect to environmental or homeostatic factors involves maintenance of internal biochemical pathways in the face of environmental perturbations, such as temperature, pH, ionic strength, nutrient availability or concentration gradients.[43,44]

Consider first the case of robustness against genetic factors: living systems are robust against parasitic takeover of the replication process. In the case of molecular replicators, parasites are species which contain the recognition motif and thereby the ability to replicate, but lack any relevant genotypic information. Life has found a way to efficiently transfer information regarding environmentally fit phenotypes over

successive generations, without being overwhelmed by parasitic growth. As experiments have demonstrated,[26,45] on way to achieve this is by compartmentalization. For example, Matsumara et al. analyzed replication by Qβ replicase in droplet-based microfluidic system and found that compartmentalization prevented parasitic takeover.[45] Even when compartmentalization is transient, it prevented the shorter, rapidly replicating parasitic species from overwhelming the replication machinery, as long as a functional replicator was selected over successive generations. In addition to the protection of genetic systems, compartmentalization can also accelerate thermodynamically unfavorable reactions through non-catalytic mechanisms. For example, Fallah-Araghi et al., demonstrated the latter in the case of a simple bimolecular reaction – imine synthesis – which was accelerated upon encapsulation in picolitre water-in-oil droplets.[46] The reaction rate was enhanced by the adsorption of reactants on the droplet liquid-liquid interface, wherein they react and diffuse back into the bulk. Other associated factors such as macromolecular crowding[47] and diffusion limitation[48] inside these compartments can also play a role in boosting reaction rates.

Biomimetic compartmentalized systems have been synthesized and studied for nearly three decades.[49,50] However, most of these systems are inaccurate replicas of living systems. Unlike living cells, the majority of these systems are either thermodynamically closed or use rudimentary mechanisms such as non-selective membrane pores,[51,52] for exchanging matter with their surroundings. The far-from-equilibrium operation of cells involves maintenance of internal variables essential for biochemical reactions that power life. These variables include, for example, pH, temperature and ionic strength. The property of maintaining internal variables, called homeostasis, is another example of robustness exhibited by living cells, as it enables them to persist and propagate despite fluctuations in environmental conditions.[41] Although homeostasis is a characteristic feature of living cells it has proven difficult to recreate in synthetic systems. Compartments that encapsulate their building blocks can synthesize these building blocks internally, and thereby self-maintain and regenerate, are considered as alive, autopoietic units.[53] This concept of autopoiesis, developed by Maturana and Varela, seeks to provide a blueprint for cellular life by defining living autopoietic units as systems possessing a semi-permeable boundary, which contain a network of reactions capable of producing and maintaining all the components of the system, including the building blocks for the boundary.[54] Inspired by this framework, chemical models of autopoiesis/homeostasis have been explored.[50,55] Although these systems demonstrated limited self-maintaining capacity, they were based on simple chemistry such as hydrolysis reactions, producing amphiphilic molecules. Furthermore, these systems did not possess any capacity for information storage or evolution. Although more biomimetic homeostatic systems have been developed subsequently,[43,56] the synthesis of homeostatic compartments capable of information storage and evolution is still a major bottleneck for the development of synthetic cells.

The consideration of Darwinian evolution and selection as a central property, and thus as a prerequisite for life, has provided answers to several questions in biology. However, as Maynard-Smith suggested, most questions in biology require at least two answers.[57] The first set of answers considers life as a collective phenomenon, with living organisms possessing heritable information, replicate and are selected based on fitness parameters. And the second set of answers envision living organisms as distinct entities which maintain structure and function by energy dissipation. This second set can be aggregated in the property of autonomy of living systems.

5. Autonomy

Living cells are self-organizing microreactors, hosting thousands of reactions. Driven by a flux of energy and matter, each living cell grows, divides and gives birth to another, thereby propagating life. They are thus, autopoietic units, that possess the means of self-maintenance and regeneration. This capacity to produce and maintain themselves is what makes autopoietic units autonomous, since there is no distinction between the 'producer' and the 'product'.[54] Realizing such systems from scratch has been proven to be very difficult. Consider for example cell free gene expression (CFE) wherein template-based replication[27] and translation into proteins has been accomplished in vitro using reconstituted components of gene expression, such as PURE[58] and cell extract-based systems.[59,60] Expression is driven by incorporating relevant metabolites and nutrients such as high-energy phosphate compounds or mono- and polysaccharides and NADH, which enable energy and cofactor regeneration.[59] Moreover, as these systems are programmed with nucleic acid-based templates, stable information storage and inheritance is possible. However, CFE systems possess limited, if any, autonomy. Living cells are continuously driven far-from-equilibrium by a flux of energy and matter enabled by membrane transporters, whereas cell-free systems tend to reach equilibrium as reactants are depleted. Moreover, while partial regeneration of CFE components has been achieved,[27,61] self-regeneration of CFE systems as an integrated whole is also yet to be accomplished due to poor expression efficiencies of most of the components[12] and the large number of proteins and RNAs involved. This is distinct from "primitive" template-dependent self-replication, which only involves copying of sequential information, whereas in case of self-regeneration, replication is coupled with macromolecular synthesis of all the biomolecular components necessary for gene expression. Typically, CFE is carried out in bulk reactions, which removes the possibility of regulatory mechanisms to counter environmental fluctuations and perturbations, and thereby homeostasis. Although CFE systems have been compartmentalized in vesicles[52,62] and water-in-oil droplets,[63] these compartments are usually thermodynamically closed and flux of energy and matter is not autonomous, i.e., it is possibly only via externally

imposed means such as manual mixing[64] or continuous flow[65] or continuous exchange reactors.[66] Lifetime of cell free expression can be extended by nutrient exchange by passive diffusion through membrane pores (with non-specific pore proteins such as hemolysin).[52,67] In fact, basic physical homeostasis has also been achieved in vesicles.[43] However, these functions are not genetically encoded[68] and have to be coupled with templated replication to resemble cellular reproduction. Finally, the amount of information encoded in the form of protein synthesis machinery far exceeds the information that is expressed or replicated from templates. In this respect, cell free systems resemble viruses rather than self-regenerating living cells.[12]

The networks of reactions in living cells have been extensively studied and elucidated, following major breakthroughs in molecular biology.[69] Despite these efforts, autonomous synthetic cells have not yet materialized. A general recognition has accompanied these developments, that the key to realizing autonomous synthetic cells, lies in understanding the far-from-equilibrium functioning of living cells.[70,71] Cells are thermodynamically open systems which continuously synthesize, transform and degrade macromolecules to sustain persistent ordered structures. Cells take up nutrients and store up chemical energy in the form of kinetically stable, thermodynamically active molecules.[72] These molecules, possess group transfer potential and undergo exergonic reactions, which are coupled to endergonic reactions, thereby driving thermodynamically unfavorable reactions such as macromolecular biosynthesis, self-assembly, transport and degradation processes.[73] Coupling with exergonic reactions of high energy molecules introduces irreversibility in these processes. This irreversibility is achieved with an energetic cost, in the form of energy loss by dissipation.[74] Endeavors to mimic this aspect of biological processes has led to considerable work in synthetic chemical fuel driven assemblies. Examples of such synthetic systems include supramolecular systems, such as molecular motors and vesicles,[73,75] and nonequilibrium soft matter systems such as active coacervate droplets, fibers and hydrogels.[76]

The importance of irreversibility has also been highlighted in a statistical physics-based framework called dissipative adaptation (DA).[71] A system driven out of equilibrium by an external source of energy or "drive" can adopt configurations that normally may not be attained solely by thermal fluctuations. It can do so by absorbing energy from the drive and dissipating it during the transition. The dissipation of energy renders these transitions irreversible. Over time, a succession of such transitions will appear as self-organization of the system into a shape that seems adapted to the prevalent environmental conditions. Hence the term – dissipative adaptation. An experimental demonstration of this principle was achieved using GTP-driven self-assembly of FtsZ protein within coacervate droplets, leading to directional growth and division of the coacervate droplets.[77] Although this study offers insights into how to design dynamic nanosystems using DA, further experimental investigations are necessary, especially in the context of information storage, rise of variation across successive generations and subsequent Darwinian selection.

6. Conclusions

To refer back to the earlier analogy of the blind men describing an elephant, so far, we know that the "beast" of life is characterized by its ability to replicate, evolve, its robustness and autonomous functioning. Perhaps there are yet more features of living systems which we will be acquainted with eventually. Moreover, significant challenges also await in the development of synthetic systems exhibiting all four features described here. Cellular function involves seamless functioning at nano-, meso- and microscale. Mirroring these hierarchies is not a simple task of mimicking specific features, but also involves achieving simultaneous execution of all different aspects of biological organization.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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