

## **Breaking the known rules of life to reveal unknown rules and therefore the limits of life**

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### **Summary**

We begin to explore the possibility that rules of life, unifying principles that explain the limits of life, exist and, if so, how they might be explored more deeply through collaboration across the sciences. Depending on the universality of the rules and the extent to which rules are hierarchically organized or context-dependent (limited to particular taxa or levels of biological organization), thoughtful manipulation of those rules to reveal mechanisms underlying the limits may expose previously unknown constraints on life. Because of the massive complexity of life, work across disciplines such as molecular biology, biochemistry, cellular biology, organismal biology, ecology and even physics, chemistry, atmospheric and geological sciences should be encouraged. Benefits include accelerated identification of previously unrecognized mechanisms or roadblocks limiting the speed of adaptation that could be exploited to produce more climate-resilient food crops, more effective disease control, and even greater appreciation for the remarkable complexity of life on Earth. Given this brief overview, we conclude with ten recommendations to facilitate further exploration and development of the rules of life.

### **What defines life?**

To understand the fundamental requirements for life, we first need to establish what life is and then explicate what rules may govern its existence. By establishing this foundation first, we improve abilities to guide studies aimed at breaking these known rules to gain insight into previously unrecognized or underappreciated rules of life. Biology is the study of life. Yet, defining life has not been easy (Margulis and Sagin 1995). For the purposes of this discussion we limit the description of life to any entity capable of self-propagation via replication of information and which uses elements to create its own structure (and therefore mass) giving it a corporeal existence that keeps itself “in” and the environment in which it lives “out.”

If life is so defined, then at least two primary rules of life emerge. To exist and replicate, energy is required. Liquid water is also assumed to be a requirement of life as we know it on Earth. In both cases, the amounts required vary across orders of magnitude along with the size and complexity of the life form. How energy requirements scale across life has been thoroughly evaluated, at least in the arena of metabolic rates, which reveals a well-known exponential relationship, where metabolism is proportional to mass to the power  $3/4$  (Brown and West 2000).

Although we suggest that two rules are the requirements of energy and water, we are unaware that anyone has yet formally called these rules of life. Evaluating the heuristic advantages and disadvantages of identifying rules of life would be worthwhile because intellectual frameworks clarify thinking and reveal directions of future research. In this vision paper, which is not currently an exhaustive literature review, we suggest that life’s need of energy and water are foundational, separating living from not-living things. These are co-requirements that then permit

the formulation of separate corporeal entities with an ability to maintain self and propagate through time. Expanding beyond these two foundational rules to define the limits and requirements for water and energy in living systems has been studied in some types of organisms by researchers in the fields of stressor biology and toxicology, as well as those who investigate extremophiles (Alpert, 2006; Doucet et al., 2009).

It is likely that, if additional rules exist, they are full of loopholes, exploited by the perpetual and persistent proliferation of biological variants whose billions of years of evolutionary experimentation provides the machinery for a remarkable ability to discover and then exploit ecological opportunities for acquiring energy necessary for replication. Interesting questions such as, How dependent on the type of organism are the rules of life?, reveal that rules of life may be context-dependent. Others such as, How dynamic through time and space are the requirements for life?, suggest that interactions of life and its environment necessitate flexible rules.

Philosophically, we suggest further thought is needed to explore if just a few fundamental rules exist or if there are millions of rules dependent on the specific tolerance of limits of each particular life form. Furthermore, these rules may be arranged hierarchically, affecting similar groups of organisms while being irrelevant to others. Therefore, articulating the exact differences between rules and simple taxon- or situation-specific limits is a necessary first step.

### **Defining the rules and capabilities of life**

Understanding the rules and mechanisms defining the limits of life, as we know it, as it has been and as it could be, provides a framework linking divergent fields of biology in the pursuit of concrete biological principles that can be applied across levels of organization. While the ability to establish biological “laws” that are concrete and unchangeable, similar to those in the field of physics, is highly improbable or impossible given the dynamic nature of biological systems, knowledge of the rules and boundaries by which life can exist would lay the foundation from which to build novel biological insight that can shape our view of life on Earth. This holistic, “outside in” concept of biological research utilizes knowledge of the definable limits of life to inform and guide new hypotheses and unearth discoveries that would not be possible from our biased viewpoint of life as it exists today.

This is particularly important given the fact that the environmental conditions on our planet are in a constant state of change. Whether driven by natural or anthropogenic factors the changing environmental contexts by which life on Earth will find itself in future years will unavoidably test the boundaries by which many life forms can exist. Defining the rules and processes that enable life on the extreme fringes of what is biologically possible will enable predictions to determine the mechanisms that may enable animals to adapt to changing environmental conditions, providing a window into what life may look like in the future. Testing the boundaries of life as it could exist is one of the core concepts driving our search for extraterrestrial life that may exist in extreme environments elsewhere in our solar system or beyond.

In addition to modeling what life may look like on Earth in the distant future, clarifying our understanding of the rules of life will expand the power of humans to modify biological systems to solve emerging problems. This includes developing new methods to improve adaptability of conservation-reliant species that would not survive without human intervention and even reviving recently extinct species (de-extinction) to improve ecosystem biodiversity and/or

function. Articulating the set of rules by which life can exist will aid in the ability to improve biological systems for agricultural production while expanding agricultural production into new, previously inaccessible environments. The potential applied applications are numerous and drive many research questions in the fields of bioengineering and synthetic biology which look to solve specific real-world problems by changing the properties of biological systems.

### **Breaking the rules?**

Why should we try to break the known or theoretical rules of life? We recognize that many disciplines have been exploring the limits of life for a very long time. Toxicology, stressor biology, biomedical sciences, agricultural sciences (e.g., pest control, nutrient limitations, and drought tolerances) and other disciplines carefully study causes of death, including exploration of the molecular mechanisms. Evaluating mechanisms of death provides evidence of the limits of life. At the known limits of life, extremophilic organisms offer information on novel solutions evolution has created to especially marginal and challenging environmental conditions. Further thoughtful study and experimentation on such organisms may pose the best chance to reveal the mechanistic limits at the biochemical and cellular levels for existence of life. Developing tools to reveal the barriers to even these extreme organisms will likely require development of new approaches. A useful step may include convening groups of scientists who assemble current information and begin to connect the dots, particularly across taxonomic groups or levels of biological organization, and attempt to identify emergent patterns across levels of biological complexity. Creating models of less complex systems, such as artificial bacteria, could provide the basis for evaluating the molecular and biochemical boundaries in whole-organism systems.

Expertise in molecular and synthetic biology, biochemistry and biophysics will be needed to determine how particular rules of life may be tested or broken intelligently. Given that organisms live in environments with other taxa, the potential and realized limits of life are expected to be manifest within the broader context of relevant biological communities where limits may be enforced by ecological and behavioral interactions, not just properties of the organisms themselves. Integrating all of this diverse and complex data will require vast new computational and systems biology approaches to assemble this information into a coherent picture of mechanisms influencing the existence and persistence of life.

Once our understanding of the limits of life are improved, and the underlying mechanisms revealed, we envision thoughtful new arenas of investigation focused on exploring acceleration of adaptation to novel situations. For example, through rapidly improving capabilities in genetics, genomics, gene editing, creation of gene drives and CRISPR technology, artificial creation of new protein designs, synthetic biology and the like, the efficiency with which we can identify the key areas needed for improvement can be hastened. With looming uncertainty regarding the rapidity with which climate may shift, for example, solving anticipated environmental challenges associated with producing food on a massive scale for nearly 10 billion humans can be accelerated.

### **Obstacles to defining the limits of life**

Building a model of the limits by which life can exist will require the coordinated interaction of both theoretical and experimental biologists. Many of the potential limits of life are not well defined and are often based on extant organisms under favorable environmental conditions. It will be critical therefore that theoretical biologists have a major role in describing what the

potential limits of life may be and how alterations in organism structure are confined within the rules that govern life in those specific context. Experimental biologists can use this framework as a guide to push organisms past their current biological limitations to test the unknown constraints of that system. This will require a large number of new technological innovations designed to identify, detect and manipulate multiple parameters as well as test new hypotheses on expanding current biological capabilities. Measuring multiple data points simultaneously would be highly beneficial to this research, since perturbation in one part of the system will likely affect multiple components that can shift the equilibrium of the biological unit.

It is important to note that when referring to the limits of life, we do not confine our definition to the most radical environmental scenarios inhabited by only the most “extreme” microorganisms, but instead rely on a much broader definition that incorporates all aspects of an organism’s key features such as body organization/morphology and physiological and cellular traits as well as how these factors influence ecological parameters. These traits set the boundaries for what is likely possible for that type and class of organism while highlighting fundamental differences in adaptive capabilities between the different levels of biological organization. Extremophiles, as defined within this broader context, can provide scientists with an important window into how life has evolved within the confines of specific biological requirements and the essential rules of life that govern and restrict organisms under those conditions. The traditional example of extremophiles are often associated with microorganisms that live in the harshest of environmental conditions found at deep sea hydrothermal vents, acidic or alkaline hot springs and even inside rocks. Similarly, multicellular extremophiles living in harsh environments such as the organisms inhabiting sub-zero polar marine environments have and will continue to make valuable model systems. Outside of these traditional extremophiles, it is important to consider other organisms as also pushing the limits of life. This could include extremely large animals, such as the blue whale, giraffe or elephant that have evolved extreme physiological traits to overcome physical challenges that restrict the size of most other organisms.

A common way of addressing unknown biological questions is by disrupting a specific trait and observing the effect of that perturbation on the larger system (i.e., cell, organisms or ecosystem). Many hypotheses can be addressed within a traditional confined or laboratory environment, however applying this approach to larger biological questions that cross many biological levels may create new ethical considerations that need to be addressed. This is particularly true when designing experiments that will create new synthetic life or engineer biological systems that will then be tested within natural biological environments and could therefore have the potential for unintended consequences that could impact target or non-target species or ecosystem structure.

The basic question of whether humans should embark on large-scale manipulation of life to better understand the rules of life will need to be addressed by a large community of stakeholders, across different ideological spectrums. The discovery of CRISPR genome editing technology, which has vast potential to modify organisms, including humans, has sparked a fierce debate over if or to what extent we should use advanced research tools to engineer biological systems. Expansion of this debate within society to establish guidelines for how biology should approach these complex ethical issues will be important for defining which rules of life should be investigated experimentally and others that may be more ethically addressed with systems models or artificial intelligence.

## **The role of reintegration**

Defining and exploring the rules of life requires communication and collaboration across all of biology, so truly does pivot on the reintegration of biology. Understanding life, how it functions and the nature of its various limits requires a deep level of knowledge across a huge array of domains of life. Such knowledge is held in collaborative groups, not individuals. In addition, boundaries of life are imposed by environment and history. Collaboration with atmospheric and geological scientists can establish a new understanding among biologists of critical constraints to evolution and life. To the extent that rules of life are also governed by, inextricably intertwined with, or subservient to laws of physics and chemistry, collaborative interactions with those specialists can also move the study of rules of life forward. Finally, breaking the rules of life to explore the possibility of discovering previously unrecognized or under-appreciated rules may be more easily done with models, so working with computational scientists can accelerate development through carefully designed theoretical experiments.

### **Conclusion and broader impacts**

The increase in big data, computational power, and technologies for manipulating biological systems are rapidly changing how we understand life as we know it. As a guiding principle for the future of biological exploration it will be important to consider the limits at which life can exist to understand the potential for life to adapt to ever-changing environmental challenges and how it might exist for future generations. To understand these boundaries and identify unknown rules of life, that cannot be revealed within the confines of known biological systems, it will be helpful to develop integrative biological infrastructure and bring our collaborative scientific spirit even more formally into place through sustaining support for interdisciplinary teams working together for years.

To move the study of rules of life forward, we recommend:

1. Clearly state the fundamental rules of life.
2. Assuming fundamental rules exist, identify the hierarchical structure of the subordinate rules and their context dependency.
3. Search for patterns in interdependency of rules permitting life.
4. Identify unexplored ways to challenge the rules of life as a mechanism for revealing previously unknown limits to life.
5. Incorporate discussions and input from all levels of biological studies, computer sciences, chemistry, biochemistry, physics and even atmospheric and geological sciences to integrate information on environmental conditions permitting life to exist, physical and chemical laws that impose known constraints on biological form and function, and currently known limits to life and solutions presented by current life at the margins of tolerance.
6. Encourage development of an academic culture that highly values cross-disciplinary interactions with immediate basic science contributions and eventual applied scientific contributions, focused on training institutions to evaluate the actual scientific contributions of individual scientists to large working groups with less emphasis on using publications and order of authorship as easy, and fallible, surrogates for evaluating individual contributions.
7. Encourage among high school and undergraduate students the appeal of deeply considering the value of broad training in the sciences with simultaneous development of one's own particular expertise.

8. Facilitate regular interactions among students and professionals to continue developing breadth of education as a life-long goal and as a way to develop common language across major disciplines.
9. Communicate to and with society about the value of understanding the limits to life, which may include predicting future responses of ourselves and important food systems to continuing environmental and climate change.
10. Anticipate new knowledge about novel innovations various forms of life may require in future environmental and climate scenarios, and promote deep and careful thought on the use of genetic manipulations to accelerate adaptation.

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