The Minimal Cell

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What You Will Learn in This Chapter

In this chapter, we will present the concept of the minimal cell and examine the reasons for this being a research goal. We will then look at two different approaches that researchers have adopted in order to create the minimal cell. We will also highlight the limits of each method and emphasize the need for the minimal cell to be clearly defined. We will define the minimal cell as any minimal system of interacting molecules that is capable of showing signs of life. By presenting several definitions of life, we will show that conventional definitions do not give practical objectives for the creation of a minimal cell. Autopoiesis is offered as an alternative, functional definition.

2.1 The Minimal Cell

There is a prevailing idea that the complexity of cells we see is the product of evolutionary processes, much like speciation. Underlying this idea is the suggestion that all cells are modifications of what is called a minimal cell. The minimal cell ought to be the simplest collection of interacting molecules that can show signs of cellular life, under specific environmental conditions. Simply put, it is the simplest possible form of cellular life, under those conditions.

While it is uncertain whether the minimal cell ever existed, or is still in existence, in the natural environment, this idea has spurred researchers into seeking to identify, or even create, the minimal cell. They do this for two main reasons. First of all, studying what constitutes a minimal cell would provide insight into the deeper principles of cellular evolution. That is, it would allow us to understand what more it will take to change simple cells into more complex ones. Secondly, a minimal cell can be augmented in various ways to confer new functions upon it. If successful, this will allow researchers to create a new and wider set of cellular tools to solve biological, medical, and environmental problems. This is like reducing a car to just the frame, axles, engine, and four wheels. On this simple base, one could build different chassis to produce cars of different types—an ambulance, a racing car, or a sports utility vehicle, say. You might even build a chassis to produce a car that can do what cars have never done before, perhaps to fly.

There are basically two main strategies employed in the search for the minimal cell: the top-down approach and the bottom-up approach. The top-down approach begins with an existing complex cell (Fig. 2.1). We then try to remove components of the cell and observe the impact of this act. If the cell stays alive, then the part removed is probably not essential to life, under those environmental conditions.

We keep doing this until we have the minimal set of components, fewer than with which, life is no longer possible. This minimal set would be the minimal cell. We will discuss the work of the J. Craig Venter Institute as an example of this approach. The bottom-up approach addresses the challenge from the opposite direction (Fig. 2.2).

Here, basic materials are put together rationally in attempts to reconstruct or mimic biological structures and behavior that might lead to cellular life. This is the sort of approach adopted by those working on chemical autopoiesis, as we will see later.

In both cases, the end point is the minimal cell. However, unless this end point is clearly defined, it will be difficult to know when the minimal cell has been produced. The major question that allows us to define the minimal cell is, "What is life?"

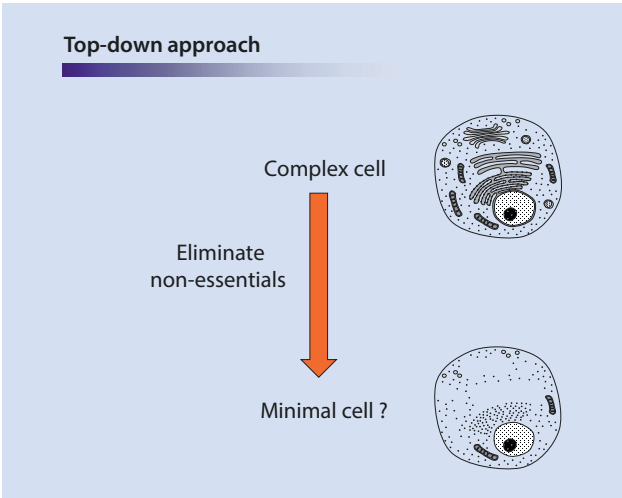

Fig. 2.1 An example of the top-down approach. A cell is systematically stripped of its components in order to attain a desired structure

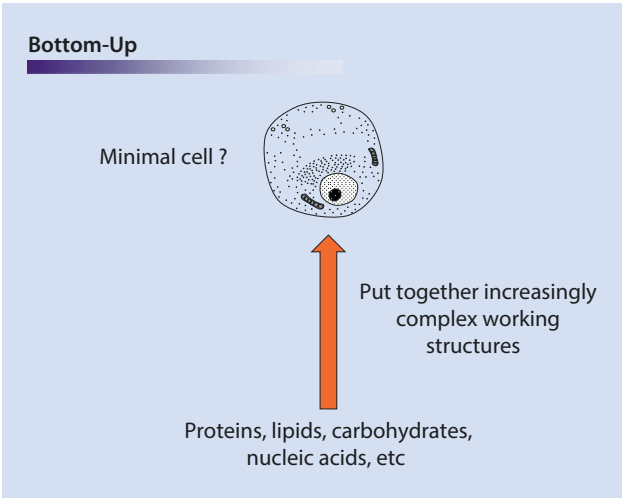

Fig. 2.2 An example of the bottom-up approach. Biomolecules can be brought together to form complex structures such as membranes and simple organelles. These, in turn, can be used to construct even more complex systems, such as a cell

2.2 Defining the Minimal Cell

This is a deeply philosophical question, like so many others that have plagued mankind through the ages. What makes this question so hard to answer is that we do not have a consensual definition for what life is. It is defined in different ways by different people with different backgrounds and different value systems. Some people adopt the same attitude that vitalism does toward the molecules of life—that life is somehow special, is outside the understanding of science, and can never be created artificially.

For the purpose of creating the minimal cell, what we need is a *functional* definition of life—one that is simple, and can be understood in terms of the basic principles of physics and chemistry. If we have such a definition, we can study and understand life more precisely. More than that, it would make the creation of artificial life possible.

When presented with a random collection of items, one can readily agree that the fly, the tree, and the mushroom are living, while the radio, the computer, and the moon are

nonliving. Even the single-celled amoeba is considered alive. But things get hazy when we look at viruses. Are viruses alive? There are some who consider them so, and they have their reasons. Others consider their dependence on a host to be proof they are not living. However, if dependence on a host is a contraindication, then should we not also consider the plasmodial parasite—or even a human embryo—nonliving? We need a more pertinent definition that will resolve such conundrums.

This need has been addressed by many philosopher-scientists. The thoughts of Erwin Schrödinger—as outlined in his book *What is Life?*—have had a great impact on both the prevailing definition of life and how people perceive the nature of life. To begin with, he stressed that life consists of phenomena that have to adhere to the laws of nature. This way, they can be understood using science and chemistry. This essentially demystifies life and makes it understandable. Schrödinger's other contribution was to propose that it was an aperiodic crystalline molecule that encoded life—that is, that such a crystal contained all the information necessary for the construction of living systems. If this were true, and if one could find and study such a molecule, one might learn what basic components are necessary for life in general. In doing so, he challenged the prevailing expectation that genetic material was proteinaceous. The year was 1944, close to the end of the Second World War.

This idea was vindicated with the publication of Watson and Crick's seminal *Nature* paper describing the structure of DNA in 1953. The paper itself focuses mainly on the structure and chemistry of nucleic acids. What was significant in Watson and Crick's paper was their description of base pairing and its crucial role in defining DNA structure. However, the truly visionary element of this paper was a comment made near the end: "It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material." With this statement, they showed that nucleic acids were capable of easily doing what proteins cannot—reproduce themselves. Naturally, it was easy to accept that nucleic acids were the basis, and hence the defining element, of life. This is the reason that some consider viruses alive, despite their dependence on hosts.

However, even this definition has its limit. How do we evaluate red blood cells, which do not have nuclei? Are erythrocytes alive? They must be somehow, because not only do they perform the critical task of transporting oxygen throughout the body; they must also be able to maintain structural integrity while repeatedly being exposed to tremendous shear stresses—not just for seconds or minutes, but for 120 days in humans.

Others attempt to describe life as consisting of eight characteristic processes: movement, excretion, respiration, irritability, growth, reproduction, adaptability, and nutrition. However, you can find exceptional examples of life where one or more of these processes are lacking, rendering this definition unreliable. Besides, this convention defines life using eight complexes processes, each of which is nearly impossible or very difficult to recreate in the laboratory. Clearly, it would not be practical to define the minimal cell on the basis of these processes alone.

2.3 Autopoiesis

The Chilean biologist-philosophers Humberto Maturana and Francisco Varela decided to develop a broader, and hence more versatile, definition of life. They defined life as a system that is autopoietic. Autopoiesis, they proposed, is characterized by (1) a system enclosed by a boundary; and (2) the enclosed system being a self-repairing mechanism.

In cells, this boundary is the lipid bilayer that makes up cell membranes. Cells themselves contain the materials and processes to produce every component of themselves, including the cell membrane. This biological factory is needed in order to replace material lost from the system in the form of waste or wear. The source of this material, or its precursor, is the environment. This means that the boundary has to be selectively permeable, allowing materials for self-construction to enter and allowing waste products to leave. An autopoietic system is, therefore, a factory whose basic function is to repair itself.

Figure 2.3 represents autopoiesis in a very basic form. There is a bounded system at the center, into which the environment provides an input. This input is processed and transformed by the machinery enclosed into materials for reforming parts of the bounded system. This process, in turn, produces an output, which then enters the environment. This basic scheme can describe most living systems, except that each component you see will be different.

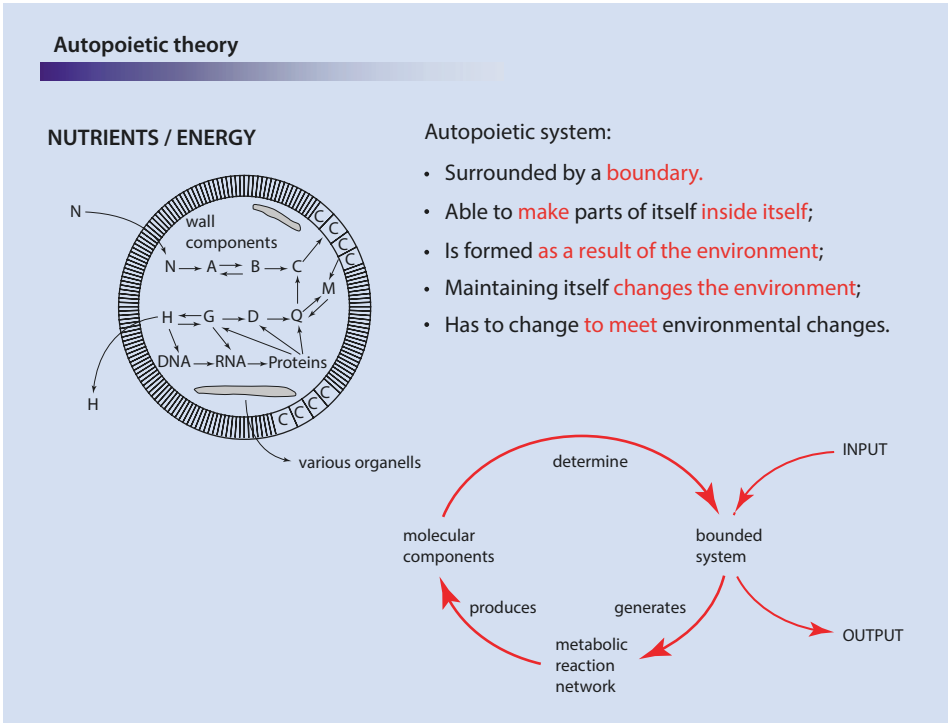

Fig. 2.3 Some basic features of an autopoietic system. These are systems that are confined by, and includes, a boundary such as a membrane. This boundary separates the interior from the surrounding environment. There is, however, exchange of material between the two spaces. Enclosed within the boundary are the components of a machine that recreates itself, including the boundary, provided it has the necessary building material. Such material enters the system across the boundary while the waste products created by the enclosed machinery are similarly able to escape. In essence, a basic autopoietic system is a self-repairing machine. The figure on the left illustrates this concept using the example of a simplified cell. (Luisi, 2003)

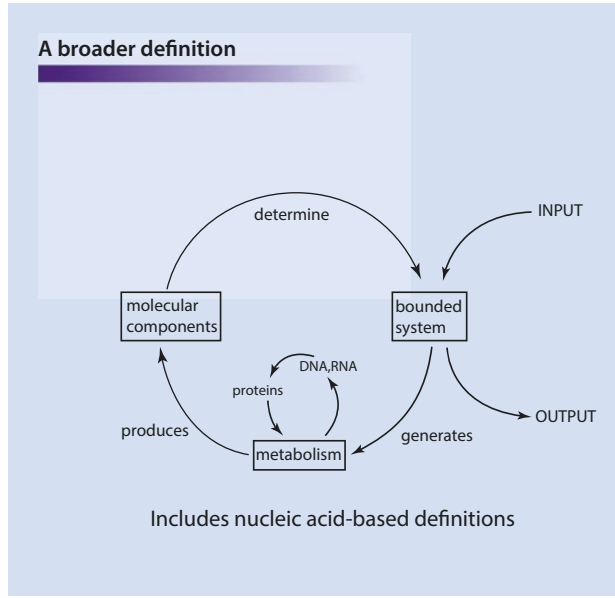

Fig. 2.4 This figure shows how autopoiesis can accommodate other biological definitions of life. In this example, it shows how it can similarly describe life based on the central dogma of molecular biology (Luisi, 2003)

For example, autopoiesis can describe a cell as an enclosed nucleic acid–based system (Fig. 2.4). Here, the factory enclosed in the boundary is the molecular biological components of the central dogma of molecular biology: DNA, RNA, and proteins. Here, the DNA produces RNA, which produces proteins, which produce the components of the entire system, using basic materials from—and expelling waste material into—the environment.

However, life is more than a status quo. So far, we have described autopoietic systems repairing themselves and maintaining their structures. Can autopoiesis also account for the growth and death observed in cells? It can if one considers that autopoiesis comprises two net processes: (1) generation of the structure; and (2) degradation of the structure. Since each process comprises complex biochemical reactions, they each have a reaction rate.

Here, simple equations allow us to describe growth, homeostasis, and death in terms of comparative reaction rates (Fig. 2.5).

When the rate of generation matches that of degradation, the system is maintaining a status quo. When the rate of generation is greater than that of degradation, the system is growing. In the case of the reverse, the system is dying. Death will occur if the system is not able to cope with changes to the environment that promote degradation.

The environment has a major influence on life. It is the initial conditions of the environment that give rise to a viable autopoietic system. As such, the autopoietic system is naturally able to use the environment to maintain itself. However, by exploiting the environment, the autopoietic system, in turn, perturbs and changes it. Sometimes, these changes are so significant that the autopoietic system also has to change in order to adapt. In this way, the autopoietic system and its environment develop together. This means that every autopoietic system and its environment share an evolutionary history. This is a cyclic process, which goes on until the autopoietic system is no longer able to cope with the new conditions. In other words, autopoietic systems are also capable of evolving.

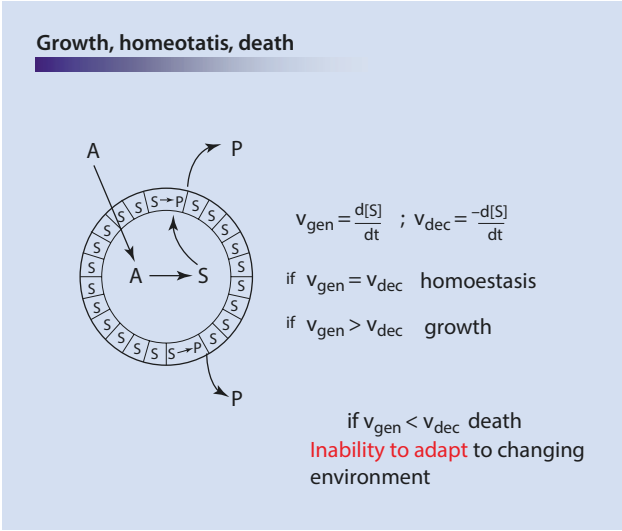

Fig. 2.5 This figure illustrates how autopoiesis can be described quantitatively. If an autopoietic system is one that is able to maintain itself in the face of constant material gain and material loss, then one can say that its anabolic processes balances out its catabolic processes. If both processes are represented by rate equations, then the rate of anabolism (v_{gen}) would be equal to the rate of catabolism (v_{dec}). When v_{dec} exceeds v_{gen}, the system would be dying. In contrast, the system would be growing if v_{gen} exceeds v_{dec} (Luisi, 2003)

Most importantly, autopoiesis provides us with clear criteria for ascertaining whether something is alive or not. These criteria can be used as engineering objectives to allow us to create autopoietic systems. The simplest, or minimal, forms of such autopoietic systems would serve as artificial minimal cells.

2.4 The Top-Down Approach: The Minimal Bacterial Genome As An Example

With this functional definition of the minimal cell, let us examine how the top-down approach was used to approximate such a system. This was work done at the J. Craig Venter Institute, founded and named after Craig Venter. His research team has been trying to identify the minimal bacterial genome, which would encode a minimal bacterial cell, under specific culture conditions.

There were two major reasons for starting with bacteria. First of all, it seems probable that, being less complex, bacterial cells might require fewer genes for encoding their structure and function. Furthermore, bacterial genomes, at the time when this work was undertaken, were among the first genomes sequenced. In fact, the first such sequencing was performed on *Mycoplasma genitalium*.

The mycoplasmas are a group of bacteria whose genomes are thought to be one of the smallest, with that of *M. genitalium* mistakenly thought to be the smallest of all. It should thus be easier to identify nonessential genes using *M. genitalium*. To do this, the research team compared the genome of *M. genitalium* with that of *Hemophilus influenzae*. They reasoned that both bacterial species must share a common, minimal set of genes that would define them as bacterial cells. All other genes would be supplementary and would code for characters that define their species, or may have other functions unrelated to maintaining life.

From this comparison, they found 250 genes that were common. These 250 genes must, they reasoned, be enough to code for a minimal bacterial cell. They were wrong, in fact. Systematic mutagenesis of these genes indicated that even these 250 included genes not essential to life. In other words, the minimal bacterial genome should actually be even smaller. In this way, they approached identifying the minimal set of genes.

2.5 The Bottom-Up Approach: Chemical Autopoiesis

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Pietro Luigi Luisi and his work on the minimal cell exemplify the use of the bottom-up approach to approximate the minimal cell. In early experiments, he employed ethyl caprylate, which is hydrolyzed at a high pH into caprylate. Caprylate is amphiphilic and hence, at its critical micelle concentration, is able to self-assemble into micelles (Fig. 2.6).

There is a clearly defined boundary, which is almost a cell membrane bilayer. Nonetheless, it isolates the hydrophobic interior from the hydrophilic environment—one of the basic requirements of an autopoietic system. Furthermore, these micelles can also entrap ethyl caprylate and hydrolyze it to caprylate through autocatalysis. In this way, the micelles are able to produce the very material from which they are made. This is, in essence, a primitive autopoietic system created using raw materials.

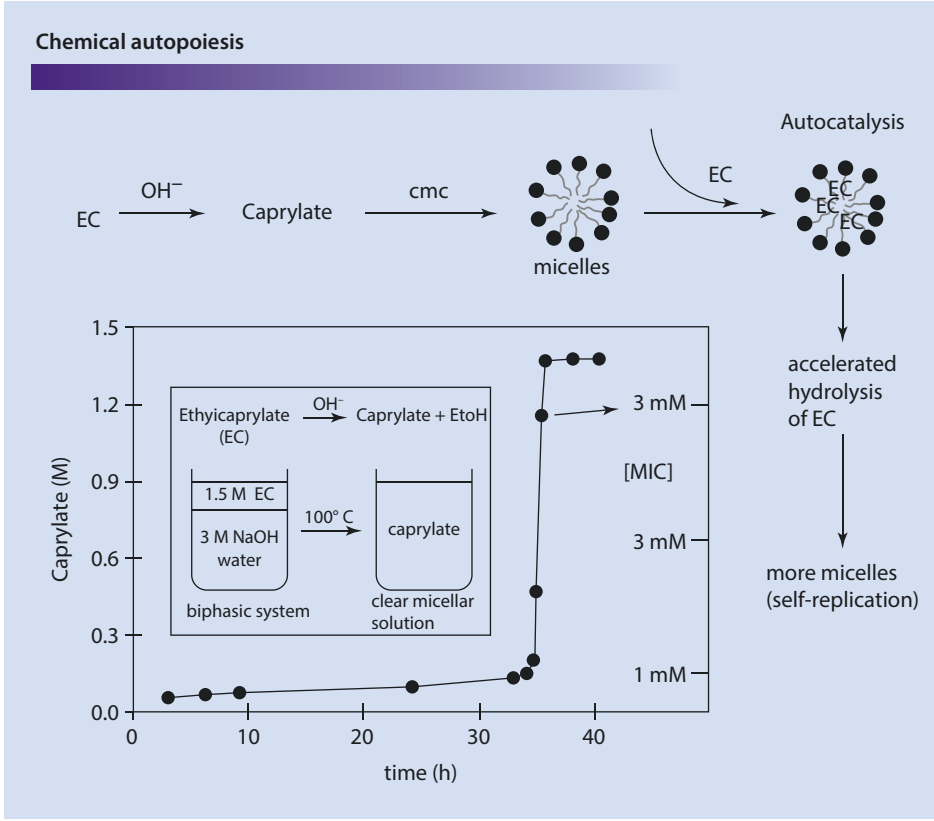

Fig. 2.6 An example of self-assembling molecules that have characteristics of an autopoietic system. Here, ethyl caprylate (EC) is hydrolyzed by a high pH into caprylate. Caprylate is amphiphilic and self-assemble into micelles which entrap ethyl caprylate. This confinement, itself, catalyzes hydrolysis of ethyl caprylate to caprylate, so contributing to the micellar boundary. In this way, a primitive autopoietic system using raw materials is generated (Luisi, 2003)

However, a micelle is not a membrane bilayer. A bilayer comprises two layers of such amphiphilic material, self-assembled in such a way that the hydrophobic domains face each other and the hydrophilic domains face either side of the membrane. This, too, Luisi has managed to emulate using surfactants. Again, he has been able to select surfactant precursors capable of autocatalysis into the self-assembling amphiphilic forms. The results are bilayered surfactant vesicles that not only can grow but also at a critical size would divide into multiple vesicles. In other words, these surfactant vesicles could not only grow, but reproduce as well! To make the system even closer to that in cells, these surfactants have also been successfully replaced with lipids that behave in a similar manner, except that they formed bilayered membrane vesicles instead. Such work demonstrates how, using only simple molecules to construct more complex structures, we are fast approaching autopoietic systems that resemble actual cells.

2.6 Autopoietic Systems and Their Environment

As mentioned, the state of the environment influences the viability of an autopoietic system. By understanding this relationship, we can understand under which conditions life will arise, under which it will thrive, and which will kill it. Clearly, any massive change to the environment, such as climate change, will present a serious challenge to autopoietic systems. How would life adapt to massive environmental changes, such as climate change?

One is tempted to assume that life is sustainable only under the conditions commonly assumed to be amenable or even critical to life. These include conditions of temperature, oxygen, and moisture. However, it is thought that the evolution of cyanobacteria, about 2.3 billion years ago, led to the sudden accumulation of oxygen in the atmosphere. Initially, this was absorbed by seabed rocks and the ocean mass, but later, it escaped into the atmosphere. Here, together with the greenhouse gas methane, it caused a massive shift in global temperatures. This is described as the Great Oxygenation Event.

Life until then was largely anoxic. The sudden appearance of highly reactive oxygen was too much for most terrestrial life to adjust to. Nonetheless, life was possible in a pregreen earth and would likely still be possible in a postgreen earth. However, it would be a different kind of life. Understanding this reminds us to consider other unconventional conditions under which autopoietic systems might arise and persist, such as conditions created in the laboratory.

This last point is particularly important where the impact of such synthetic organisms on existing life is concerned. One way to ensure that synthetic cells do not overwhelm or replace existing flora or fauna is to ensure they cannot thrive outside the laboratory nor interact with and modify existing life. Creating autopoietic systems that can persist only under non-natural conditions would be one way to control the spread and growth of synthetic organisms. This concept will be re-examined in the last chapter.

Take-Home Messages

1. The minimal cell describes the simplest possible form of cellular life, under a specific set of conditions.
2. A minimal cell can be modified to create more complex cells or cells with special functions.
3. This process would allow us to learn how simple cells might evolve into complex ones.
4. Researchers use either the top-down or bottom-up approach to try to create the minimal cell.
5. For this to be practical, the minimal cell must be defined practically.
6. Autopoiesis provides a functional definition of life and, hence, of the minimal cell.
7. Autopoiesis is the ability of a membrane-bound system to use material from its environment to produce or repair all parts of itself, including the membrane boundary. In the process, any waste material is released into the environment.
8. Autopoiesis is made possible by the environment and, in turn, affects the environment.
9. The ability of an autopoietic system to adapt to changes in the environment determines whether it will thrive.
10. Creating autopoietic systems that can thrive only in non-natural environments is a means to reduce any undesirable impact such synthetic organisms would have on existing life.

Further Reading

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