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# Building artificial cells and protocell models: Experimental approaches with lipid vesicles

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**Lipid vesicles are often used as compartment structures for preparing cell-like systems and models of protocells, the hypothetical precursor structures of the first cells at the origin of life. Although the various artificially made vesicle systems are already remarkably complex, they are still very different from and much simpler than any known living cell. Nevertheless, the preparation and study of the structure and the dynamics of functionalized vesicle systems may contribute to a better understanding of biological cells, in particular of the essential features of a living cell that are not found in the non-living form of matter. The study of protocell models may possibly lead to a better understanding of the origin of the first cells. To avoid misunderstanding in this field of research, it would be useful if generally accepted definitions of terms like ''artificial cells,'' ''synthetic cells,'' ''minimal cells,'' ''protocells,'' and ''primitive cells'' exist.**

**Keywords:** artificial cells; liposomes; minimal cells; origin of life; protocells; synthetic cells

## Introduction

All known forms of life are structurally and organizationally amazingly complex, independent of whether one thinks of highly developed multicellular organisms or simpler unicellular life forms like yeast or bacteria (Fig. 1).[1–3] For all forms of life as we know it today, a cell is the basic unit. Every organism either consists of cells or is itself a single cell. Cells are the smallest units that exhibit the property of life. Understanding the structure, function, and the dynamics of cells at the molecular levels is generally believed to be a prerequisite for an understanding of the cells' key features that differentiate the living from the non-living.[1–3]

Following the lines of thinking of the American physicist Richard P. Feynman (1918–1988) about the relation between the understanding of something and its making – ''What I cannot create, I do not understand'' – a real understanding of a living cell would imply that one is able to create it. In other words, if one understands how living cells are built, how they work, and how the different components of the cells are dynamically interlinked, one should in principle be able to synthesize a cell from scratch, *i.e.*, one should be able to assemble a functional living cell *ab initio* from its non-living components. Currently, this seems to be on the ultimate agenda of synthetic biology.[4,5]

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## Some of the currently known key features of living cells[1–3]

To illustrate the complexity of living cells (Fig. 1), some of the key features of all living cells are listed:

(i) all cells have a plasma membrane, which separates the interior of the cell from the environment, and which controls the passage of nutrient molecules into the cell and the passage of waste out of the cells;
(ii) all cells store their heredity information in the form of double-stranded DNA;
(iii) all cells use similar molecular mechanisms for transcribing and translating the genetic information encoded in the DNA;
(iv) all cells use catalytically active proteins (enzymes) to catalyze chemical reactions inside the cell, the sum total of these reactions being the cell's metabolism;
(v) all cells are ''biochemical factories'' that transform nutrient molecules *via* metabolic pathways into components of the cell leading to cell growth, to a copying of the heredity information and finally to cell division;
(vi) all cells are self-regulating systems that are able to respond to certain stimuli;
(vii) the shape of cells depends on their function and can vary considerably, as does the size of cells, which is often 1–5 $\mu$m in the case of bacteria, and 10–30 $\mu$m (and more) in the case of eukaryotic cells; and
(viii) all known cells use the ''same type of chemistry'' and many processes inside the cell use the same type of molecules (*e.g.*, water, ATP, citric acid, DNA, proteins, *etc.*), among which the macromolecules are composed of the same set of building blocks.

## Artificial cells

If one uses the term ''artificial'' to indicate that something is ''man-made,'' ''produced rather than natural,'' then an artificial

  

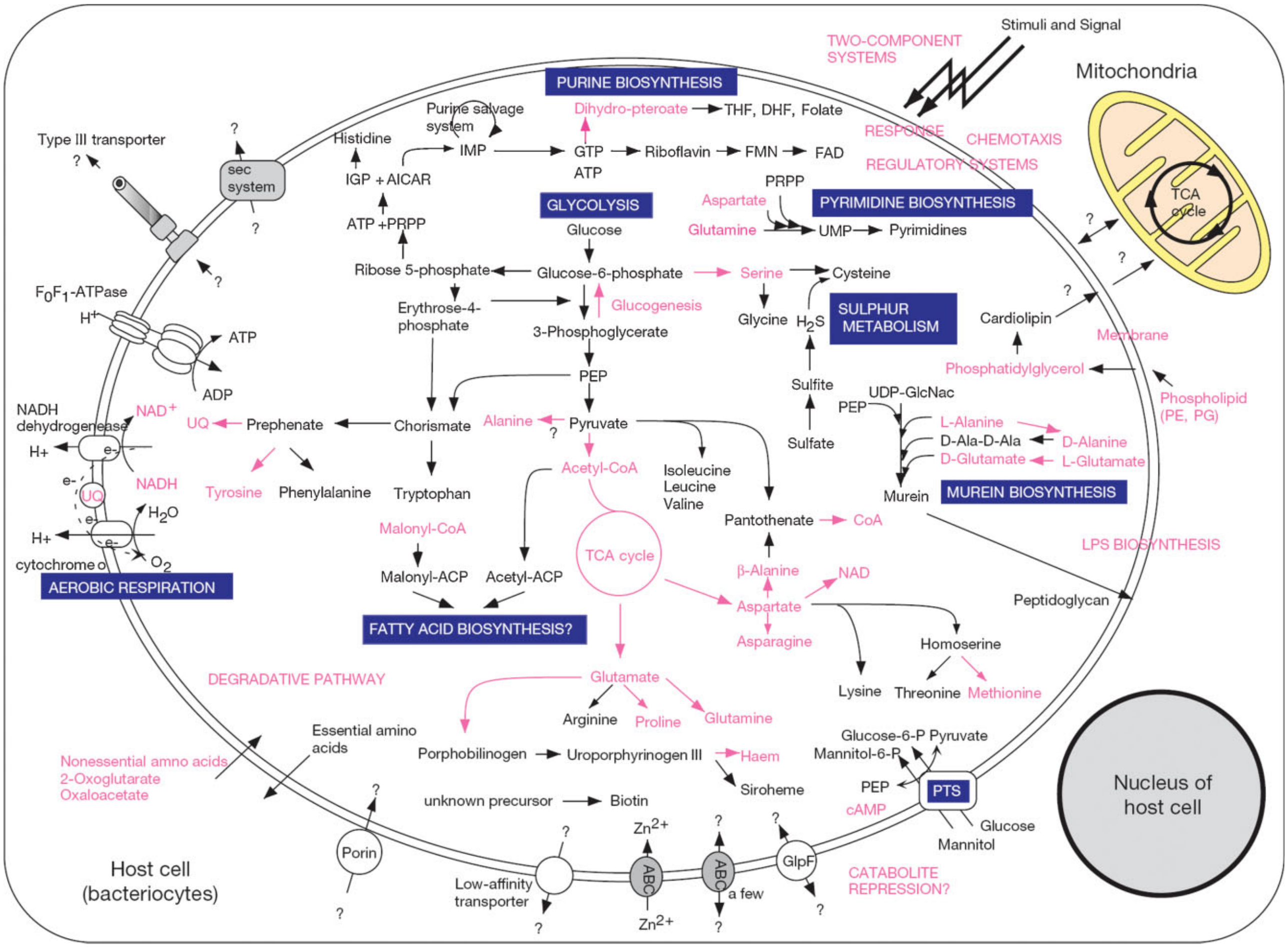


**Figure 1.** Schematic representation of the metabolism in the bacterium *Buchnera* sp., which lives as symbiont in cells of aphids (small insects). Reprinted from[83] with permission from Macmillan Publishers Ltd.

cell would be a man-made cell. The question is then how the term cell is used in this context. If the term "cell" stands for a system that has the characteristic properties of currently known "living" cells (see above), then an artificial cell would be a "man-made living cell." Therefore, preparing an artificial cell would mean making life! Since "synthetic" is synonymous to artificial, "artificial cell" and "synthetic cell" express one and the same entity. This definition of artificial cell can be compared with the meaning of synthetic (artificial) vitamin C. The molecule called "vitamin C" [L-(+)-ascorbic acid] has a defined and unique chemical structure. The name vitamin C stands for the chemical structure of the molecule. From a chemical point of view, there is no difference between synthetic vitamin C and natural vitamin C.

The problem is, however, that in literature and in daily life, the use and the meaning of the terms artificial and synthetic in connection with a structure or system often are related to the complexity of the structure or system one is tempted to make artificially. If one speaks about artificial flowers, nobody thinks of real flowers. Artificial flowers may be made from synthetic polymers or paper. They just look like flowers, but nothing more. Furthermore, the meaning of artificial or synthetic may change over the years. This is illustrated with the following example. Insect silks are made from proteins. Decades ago, at the time of the industrial polymer chemist Wallace H. Carothers (1896–1937), the synthesis of proteins, in particular silk proteins, was not possible. Furthermore, the precise structures of the silk proteins were not known and the (bio)technologies to synthesize proteins were not yet developed. Therefore, "artificial silk" was at that time a material that resembled silk – called nylon – but which was structurally rather different from silk. Although nylon and silk proteins are both polyamides, the chemical structures of the two types of macromolecules are very different. There are, however, some similarities in the mechanical properties between natural silk fibers and fibers made from nylon. Today, the structures of silk proteins are better known and the preparation of these proteins is possible, mainly thanks to the developments in analytical and synthetic chemistry, molecular biology, biotechnology and bioengineering. Today, artificial silk fibers can be made as material that is composed of the same type of protein molecules found in natural silk, made at will by man, although the preparation of a synthetic silk that is identical with nature-made silk is still not possible.[6,7]

In the case of living organisms – even unicellular bacteria as examples – the complexity of the system is so high (Fig. 1) that the "bottom-up synthesis" of these systems is currently not possible, and the use of the term artificial is often more in the sense it was used decades ago for the artificial silk prepared at that time.

## General considerations about building artificial cells

Whether the synthesis of artificial living cells will be possible in the near future, depends on at least two factors, as outlined in the following.

First, the ability to synthesize a living cell depends on the structure and complexity of the components from which the synthetic cell should be made, (i) whether the components are individual molecules, DNA, enzymes and other proteins, lipids, *etc.*; or (ii) whether the components – or some of them – have already a (nature- or man-made) complexity which is close to the complexity of a living cell. If a synthetic cell was made from individual molecules only, this would be a bottom-up synthesis, a "chemical approach,"[8] or an "*in vitro* synthetic biology project."[9] This can be compared with the construction of a contemporary functional automobile, *i.e.*, a motorized road vehicle; the difficulty of correctly assembling the automobile's components into a functional car certainly depends on whether one starts with the smallest parts from which the automobile is built, *i.e.*, the hundreds of different pieces, or whether one starts for example with two components only, an engine and an automobile from which the engine is missing. In this latter case, one just needs to correctly assemble the two pieces together to obtain a functional car, which is easy if one knows how to correctly connect the engine with the rest of the car. Of course, making the analogy between a mechanical object and a living biological cell is attractive but may at the same time be misleading. Living systems are the result of emergent properties,[10] *i.e.*, new properties arise that are not present in the parts from which the living system is made and derive from the collective behavior of the system, which in turn affects the dynamics of its parts. The emergent properties can not be predicted by knowing the properties of the parts. This is very different in the case of a car, which is designed to perform a specific task and where all properties and functions are predicted and controlled by the designer. The cell has no "designer" but has been shaped by evolution, which kept those structural and functional units that were internally coherent. The dynamic nature of a living system, the structural and regulated temporal interdependencies of the many individual components inside a cell, and the exchange of molecules with the environment make it difficult to prepare a stable non-living structure from a living cell by removing "life-supporting" parts of the cell, for instance the genome, to obtain a structure which could be compared with an automobile without engine. This latter example is related to the experiments with bacterial cells reported by the J. Craig Venter Institute. The entire genome has been transplanted from one type of cell to another type of cell,[11] and it is even likely that a man-made genome can be transplanted.[12] Remarkably, the complete chemical synthesis of a bacterial genome has already been achieved,[13] and it may soon be possible to insert such an artificial genome into another type of bacterial cell to produce a new bacterial organism that is under entire control by the synthetic genome. In the transplantation experiments described by Latigue *et al.*,[12] the genome from a bacterial cell was incorporated into yeast cells in which the bacterial genome was modified. Afterward, the modified genome was removed from yeast cells and again incorporated into bacterial cells.[12] The recipient cells, however, were not identical with the donor cells since the donor cells from which the genome was extracted were destroyed during the gene removal process. Therefore, the entire manipulation, as remarkable as it is, should probably be considered as a modification of living cells and not a synthesis of a living cell from non-living components. The recipient cells were always alive during the entire transplantation process. These types of experiments belong to *in vivo* synthetic biology projects.[9]

Second, the possibility of preparing a synthetic cell from its components depends on how one specifies the properties the cell must have. If the considered synthetic cell fulfills all the generally accepted key properties common to all currently known living cells, then one could claim that the synthesized cell would be a living synthetic cell. In this case – independent of the nature and complexity of the non-living components from which the cell is built – the successful preparation of the cell would actually mean that life could be synthesized from the non-living. If the system prepared had only some, but not all, of the features of a living cell, the system would be different from a living cell and should, therefore, not be called synthetic cell. Again, compared with an automobile, a vehicle without wheels is not an automobile since all automobiles – by the chosen definition – have wheels. This vehicle without wheels may, however, have some of the parts of all automobiles, *e.g.*, a steering system, an engine or brakes. Even if the car had all the necessary parts to be a car, there is, of course another important difference in the features of a car and a living cell. The car only properly functions if a driver, or an external control system, controls the parts of the car, its speed, change in direction, *etc*. In the case of a living cell, the cell itself has the control over all parts and functions.

This simple and not unproblematic comparison with automobiles may help in better defining what is usually meant in literature when synthetic or artificial cells are described. A generally accepted definition of artificial or synthetic cells, and a strict use of such a definition, would be very useful, and would help in avoiding confusion and misunderstanding in this field of research.

The meaning of "artificial cells" or "synthetic cells" has partially changed over the years due to scientific developments and theoretical and philosophical considerations of the living. If artificial cells are defined as "spherical ultra thin membranes of cellular dimensions enveloping biologically active material;"[14] or "engineered particles that are roughly the size of biological cells, and have engineered functionality that is meant to mimic some aspect of cell behavior;"[15] or "water-insoluble man-made particles that can perform a specific biological function in the body without being recognized by the defense system;"[16] or liposomes,[17] these types of artificial cells have not much in common with a living cell. The same is true for synthetic cells if this term is used for a lipid vesicle system with an encapsulated poly(ethyleneglycol)/dextran aqueous two-phase system.[18]

Apart from all these examples that do not have much in common with a living biological cell, there is another aspect to consider. If one were able to prepare a system that had all necessary overall structural and dynamic characteristics of a living biological cell – but if this system were built from components that were at least partly different from the types of components present in biological cells[19] – then such a system could be called "artificial living system."

The term "semi-synthetic minimal cells" has been coined for cell-like structures that are composed of a minimal number of components necessary to ensure the key features of a living cell: self-maintenance, self-reproduction, and the possibility to evolve (Fig. 2).[8,10,20–23] The critical issue here is, of course, whether everybody agrees with these particular features as sufficient characteristics of a cell system to be called living. As pointed out by Deamer,[24] "there are many kinds of cells which are alive – for instance adult neurons – which never divide or evolve." Most importantly, since there is still no general agreement about the definition of life,[25–28] it is useful if one always specifies how the terms "life" and living are used in a given context, *e.g.*, in the context of the synthesis of life,[29] or in the context of the synthesis of non-biological "living systems."[19] If one defines life as a "self-sustaining chemical system (*i.e.*, one that turns environmental resources into its own building blocks) that is capable of undergoing natural selection,"[28] then the presence of genetic material (nucleic acids or nucleic acid analogs) and Darwinian evolution in synthetic life forms is a prerequisite.

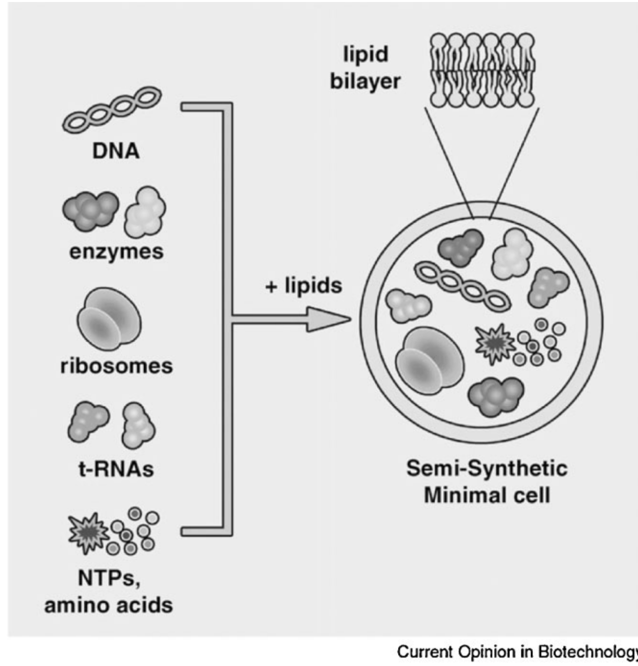


**Figure 2.** Schematic representation of a "semi-synthetic minimal cell" as defined by Luisi and coworkers. Reprinted from[8] with permission from Elsevier.

## Minimal cells

There is no general agreement about the definition of a minimal cell. Often, a minimal cell is defined as a cell that has a genome that is as small as possible.[30] Symbiotic bacteria are existing cells that have very small genome sizes (Fig. 1). They live from complex nutrients that are delivered by the host cells; therefore, these nutrients do not have to be synthesized by the bacteria themselves. Minimal cells according to this definition are cells with a minimal set of genes encoded in DNA. The general approach toward the preparation of such types of minimal cells is to start with an existing organism, *e.g.*, a bacterium, and to try to eliminate as many genes as possible to arrive at an organism that is still alive in the particularly chosen environment ("top-down approach").[30] So-prepared minimal cells should be considered as modified existing cells and not synthetic cells in the sense discussed above. These types of "minimal cells" are the results of bioengineering of existing cells, experiments which are called by Forster and Church[9] *in vivo* synthetic biology projects.

As mentioned above, the term "minimal cell" is also used as part of the term "semi-synthetic minimal cell," to describe a cell-like system that has a minimal but sufficient number of components to display specified and defined minimal properties of living cells (self-maintenance, self-reproduction and possibility to evolve), see Fig. 2.[8,10,20–22] The chemical structure of the components and the semi-synthetic minimal cell's metabolism may be different from the chemical structures and metabolism of any of the known living cells. Similarly, the term minimal cell has also been used for "a membrane-bounded construct that can self-maintain, self-reproduce, and evolve."[31] Again, there is a need to clearly specify how the term minimal cell is defined, if it is used in literature, just to avoid confusion and misunderstanding.

## Protocells

In the field of research into prebiotic chemistry and the origin of life, protocells are generally defined as hypothetical precursor structures of the first cells, which are assumed to have been formed at the origin of life about 4 billion years ago.[32–35] Therefore, protocells are structurally and organizationally simpler than biological cells and may be totally different from any known biological cell. On the time axis of the history of Earth, protocells are placed before the appearance of the first cells.[33] This means that protocells – if they existed – were composed of chemical components that do not require living systems for their synthesis. It should therefore in principle be possible to synthesize all the components of a hypothetical protocell under plausible prebiotic conditions, whatever these conditions were.[36–38] Furthermore, it is assumed that the transition from protocells to the first cells was the key step in the transformation of non-living to living matter.[10,33,34]

The term "protocell" has also been defined as "a cell-like construct fabricated experimentally that exhibits some, but not necessarily all, traits of living cells."[31] This definition of protocell includes the possible prebiotic precursor structures of the first cells, but also includes other possible systems based on molecules and chemistries that most likely did not exist in prebiotic times.

## Experiments with lipid vesicles for the preparation of artificial cells and protocell models

The "bottom-up" preparation of an artificial cell – defined here as a system with all the known characteristic properties of a contemporary biological cell (see above) – by assembling individual molecular components is not possible today. The same is true for the "bottom-up preparation" of a minimal cell – again this minimal cell being defined in the sense of a cell as we know it today, equipped with a minimal size genome (see above).

However, all attempts toward building an artificial cell are aimed at mimicking at least some of the properties of living cells. During the last couple of years, the structure and dynamics of these cell-mimicking chemical systems have become more and more complex. Furthermore, in the majority of cases, lipid vesicles (liposomes) – particularly unilamellar lipid vesicles – were and are used as compartment structures. The reason for this is simple: (i) the boundary of a unilamellar lipid vesicle is a bilayer of amphiphilic lipid molecules, similar to the lipid matrix of the plasma membrane of all known cells; (ii) there are established methods for preparing lipid vesicles with sizes in the range of the sizes of cells;[39–41] and (iii) lipid vesicles can undergo shape changes, budding and fission, similar to biological cells.[42–44] It is therefore straightforward to use vesicles as convenient cell-mimicking "containers" for carrying out more and more sophisticated reactions that typically also occur in biological cells, or that resemble reactions occurring in cells. Among the many attempts that have been summarized in several reviews,[8,10,20,22] only a few are mentioned here: (i) enzymatic synthesis of nucleic acids inside lipid vesicles;[45,46] (ii) DNA amplification with the polymerase chain reaction inside lipid vesicles;[47] (iii) protein expression inside lipid vesicles;[48–54] (iv) protein expression inside lipid vesicles of a protein that makes the vesicle membrane more permeable to allow more efficient nutrient uptake (Fig. 3);[55,56] (v) replication of genetic information with self-encoded replicase inside lipid vesicles;[57] and (vi) autocatalytic sugar synthesis (formose reaction) inside lipid vesicles.[58]

As mentioned above, protocells are simpler systems than contemporary or early cells, although they do resemble cells. Since chemically simple vesicle-forming amphiphiles are known, including amphiphiles that can be synthesized under presumably prebiotic conditions, it is obvious that vesicular compartments are currently often also considered as possible protocell models. The amphiphiles include fatty acids,[59–63] and polyprenyl phosphates.[64,65] Research in this field has made considerable progress during the last few years[66–69] with the recent remarkable achievement of a template-directed synthesis of a genetic polymer inside potentially prebiotic vesicles (Fig. 4).[70] As pointed out by Deamer,[24] the general challenge is to develop a specific functional system of compartments which is specifically regulated.

What is missing so far, in all the reactions occurring inside cell-mimicking vesicle containers, is a direct link between the reactions and a growth and reproduction of the vesicles.[29,71] For this to happen, amphiphilic molecules need to be synthesized either from water-soluble precursors only, with a C–C bond formation chemistry in aqueous solution, or from water-soluble and water-insoluble precursors. In the latter case, such a vesicle-containing system should be capable of accommodating substantial amounts of water-insoluble molecules to achieve the synthesis of the required amounts of amphiphiles for observing vesicle growth and reproduction.

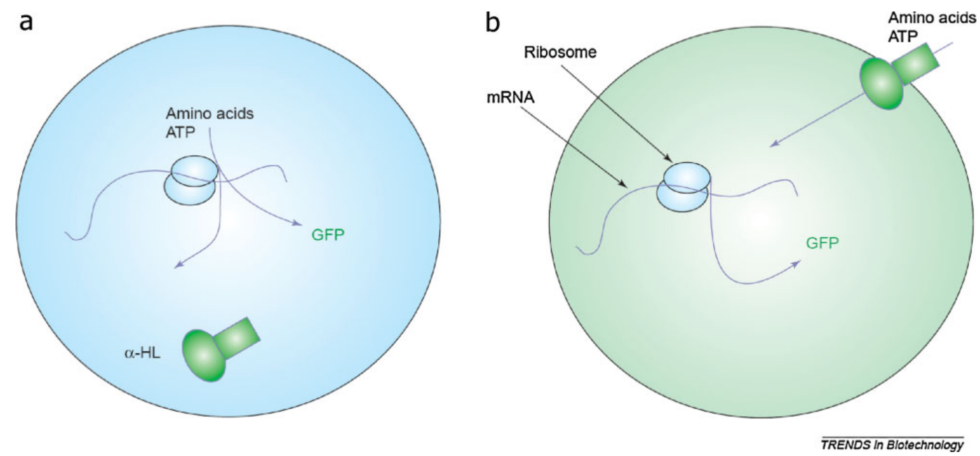


**Figure 3.** Schematic representation of gene expression within a lipid vesicle. The two proteins α-hemolysin and green fluorescent protein (GFP) are expressed inside the vesicle. The experiments were carried out by Noireaux and Libchaber.[55] Reprinted from[56] with permission from Elsevier.

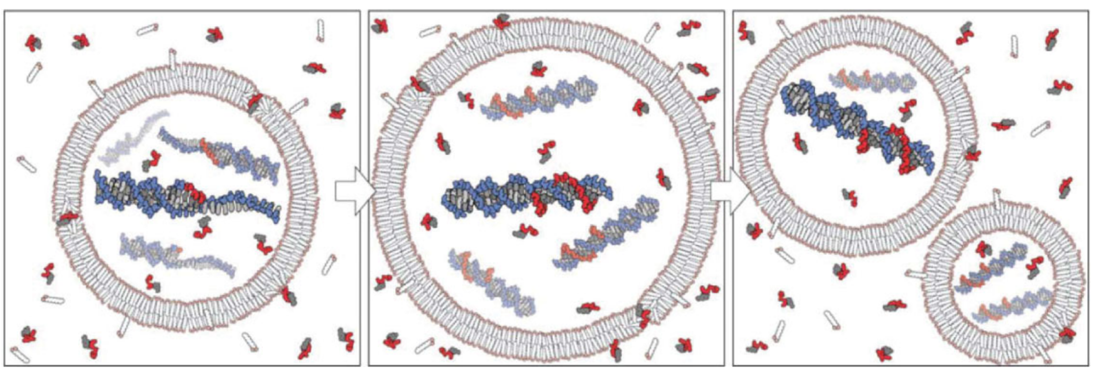

**Figure 4.** Schematic representation of a vesicular protocell model, which includes membrane growth and template copying inside the vesicles. The template-directed synthesis of a genetic polymer could be achieved inside the vesicles Szostak and coworkers. Reprinted from[70] with permission from Macmillan Publishers Ltd.

## Conclusions

Apart from the problem of not having a generally accepted definition of artificial cell, all the vesicle systems studied in relation to the construction of cell-like structures with as many properties of a living cell as possible are still far away from being something close to a biological cell. However, the experiments carried out with these vesicular cell-mimicking systems may contribute to a better understanding of how cells work and to a clearer definition of systems that are at the transition between the non-living and the living. A clear definition of the various terms mentioned in this essay would greatly help in advancing progress in this highly interdisciplinary and fascinating field of research. The various investigations of cell-like systems may at some stage result in artificial reaction systems that find their use in analytical or medical applications, or in biotechnology for the production or transformation of organic compounds, *i.e.*, as small vesicle-based cell-mimicking biochemical factories.[23,72–75] The research on protocells or – "primitive cells"[76] – in connection to the question of the origin of life is directly linked to the "origin of systems."[24] As pointed out by Deamer,[24] living cells are "a complex set of molecular components that interact in order to carry out a specific function, regulated by a range of control mechanisms." The challenge is to develop a control system that regulates the function.[24] Even if such a system was developed with molecules and reactions that were not of prebiotic relevance, it would already be a big achievement. A broader theoretical framework for these type of chemical systems can be found in the work of Gánti on the "chemoton"[77] and in the work of Maturana and Varela on "autopoiesis".[78,79] The implementation of these concepts into experimental chemical systems is one of the guidelines and the general framework of the research area of "Systems Chemistry."[80–82]

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