A goal of biochemical-based artificial life is to synthesise a minimal living cell from bottom up. Vesicles seem the most convenient basic building block of such a cell, for they are able to self-assemble and self-reproduce. In this seminar vesicle fission is presented and a possible path for synthesising an artificial cell is described.
1 Introduction

Artificial life (also known as ‘ALife’) is a field of study that connects several different natural and social sciences. It is a discipline that studies life and life-like behaviours: growth, adaptation, reproduction, learning, death... Its main goal is to synthesise life. There are three kinds of artificial life, each of them trying to recreate different aspects of life. ¹

Software-based artificial life (Soft ALife) creates digital constructions and simulations that exhibit life-like behaviours. ¹ The most well-known example of Soft ALife is the Game of Life. ² It was developed by John Horton Conway in 1970 and it is not a typical game. The only input asked of a player is to set the initial state, which determines system’s development in the future. The system is a two-dimensional orthogonal grid and each square represents a cell that is either dead or alive. The development of a system is set by four rules:

1. Any live cell with fewer than two live neighbours dies, as if caused by under-population.
2. Any live cell with two or three live neighbours lives on to the next generation.
3. Any live cell with more than three live neighbours dies, as if by overcrowding.
4. Any dead cell with exactly three live neighbours becomes a live cell, as if by reproduction. ²

In each step, the rules are applied to all cells and therefore a new generation is formed.
The second kind of artificial life is hardware-based (Hard ALife). It produces hardware implementations of life-like systems. Examples of hardware based artificial life are autonomous robots.

In this seminar we focus on the third kind of artificial life, the biochemical-based artificial life (Wet ALife). It is trying to synthesise life from biochemical substances. Its ambition is to build a living cell. Not a cell as complex as cells of today’s living organisms, but the simplest cell that can be considered alive. It must be made of a collection of molecules, simple enough to form by self-assembly, but still complex enough to exhibit properties of a living organism. An important question is what it means for a cell to be alive. Due to its diversity defining life is extremely difficult. A possible definition for a simple cellular system is that it is alive if it can self-reproduce and if it is subject to Darwinian evolution. Rather than explaining and synthesising life-as-we-know-it, artificial life is about life-as-it-could-be. The real question is therefore not how a biological system functions, but rather how it could in principle function with a minimal set of elements.

This last branch of artificial life is very closely related to research of origins of life. It is known how a cell functions but that does not give us the answer on how life on Earth started. Eukaryotic and even prokaryotic cells are very complex and are a product of billions of years of evolution. If we tear the simplest bacterium apart and take away all but its most essential components, we are still left with a very complex structure, containing hundreds of genes, thousands of proteins and other molecules. That is why to get a cell that contains only what is essential for life, we must build from bottom up. In this sense artificial life is a tool for research of origins of life.

Life can be considered as a sum of two replicating systems: information system, such as genome, that enables Darwinian evolution and a structure in which it resides. The simplest way is to begin with a nucleic acid genome, as RNA and DNA are the only currently used genetic materials. RNA is the simpler of the two, and that is why it is frequently considered to be the protocell genetic material. For the compartment in which the genome resides, the most obvious choice is a vesicle of lipids or similar molecules. Two things speak in favour of vesicles: lipid bilayers that compose vesicles also compose membranes of today’s cells and vesicles are able to grow and self-reproduce in various ways.

## 2 Vesicles and vesicle fission

Every known cell is confined with a membrane made of amphiphilic molecules. These are molecules that have both hydrophobic and hydrophilic parts, for example phospholipids, glycolipids and fatty acids. Because of their amphiphilic nature, these molecules assemble and form micelles, bilayers or vesicles when dispersed in water (Fig. 1a).

Micelles are very small. They are balls of amphiphilic molecules, in which hydrophilic heads are turned outside, towards water solution, and hydrophobic parts are turned inside. A bilayer is a membrane in which hydrophobic tails are turned facing each other and hydrophilic
heads are turned outside facing the water solution. Because of unfavourable high energy at the edges, the bilayers preferably turn to close upon themselves and form vesicles.\(^7\)

A vesicle is an enclosed space, separated from the surrounding environment with a permeable membrane made of amphiphilic molecules. In a unilamellar vesicle, membrane consists of one bilayer only, whereas in a multilamellar vesicle the membrane is made of several bilayers.

**Figure 1**: a) Micelle is a small ball of one layer of amphiphiles, its hydrophilic parts facing the outside. Bilayer sheet consists of two layers of amphiphilic molecules facing each other with hydrophobic tails and facing the outside with hydrophobic heads. A liposome is a vesicle made of a closed lipid bilayer.\(^6\) b) Spontaneous curvature \(C_0\) is a property that describes shape of a molecule.\(^8\)

Vesicles are probably the most important topic of biochemical-based artificial life. They are able to self-reproduce and their composition is very similar to a cell membrane.

Whether certain molecules will form one of the previously mentioned structures and which one they will preferably form, depends on spontaneous curvature of the molecules (Fig. 1b). Spontaneous curvature of a molecule is a property that describes how a molecule is shaped. If a molecule is cylinder-shaped (the head is as wide as the hydrophobic part), its spontaneous curvature \(C_0\) equals zero. For conic shaped molecules, where the head is wider than the hydrophobic part, \(C_0\) is positive. For inverted-cone-shaped molecules, \(C_0\) is negative. Structures are formed only for values of \(C_0\) close to zero. If \(C_0\) is positive, the structure of a micelle is preferred.

Noireaux and Libchaber\(^9\) performed an experiment, showing that enclosing molecules into a small volume can have important applications for chemical reactions. An *Escherichia coli* extract was used to perform *in vitro* transcription and translation, which are the two universal steps of genetic expression. The protein expression lasted for 2 hours when the cell-free expression extract was free in a feeding solution. In second part of the experiment the cell-free expression system was enclosed in a vesicle, forming a bioreactor, and the vesicle was put into a feeding solution. This time the protein expression lasted for 5 hours and much more proteins were produced due to the osmotic pressure. Compartmentalisation obviously acts beneficially in the process of protein expression, which is one of the most important processes happening in living organisms. Possibly, this is why compartments are needed for life in the first place.
Vesicles may well be considered as predecessors of the first living cells, as they are able to self-reproduce, are similar to today’s cell membranes and are closed compartments that are probably needed for life to exist. The first membrane systems in early life are commonly believed to have been much more unstable and permeable and also chemically more primitive than today’s cell membranes.

Vesicles are capable of growth, fusion, fission, budding, internal vesicle assembly and vesicle-surface interactions (Fig. 2). For the process of self-reproduction to start, a vesicle must first grow. Growth can be accomplished by incorporation of free amphiphilic molecules and micelles into the membrane or by fusion with another vesicle. When the vesicle grows enough it divides into smaller ones. This division can happen in different ways.

![Figure 2: Models of vesicle replication. Model I represents vesicle fission through discrete steps. Growth is accomplished by incorporation of free amphiphiles, micelles and by fusion with other vesicles (path A) or by precursors that are chemically modified to become membrane components (path B). Paths C and D show vesicle fission by budding that can result in more or less equal daughter vesicles. Model II represents internal vesicle assembly, daughter vesicles are then released. Model III represents vesicle-surface interactions that can result in vesicle fission.]

2.1 Matrix effect

If amphiphilic molecules are put into water solution, vesicles are formed, which have a very broad size distribution. However, if some vesicles of given size are present in the water solution at the beginning, the rate of vesicle forming increases and final vesicles are all in size close to the seed vesicles. The size distribution is much more homogeneous. This is called the matrix effect. It is an interesting phenomenon which demonstrates that existing bilayers have an important role in the formation of new vesicles. It does not show however how new vesicles come into being. Only experiments with electron microscopy were able to demonstrate that new vesicles form by division of seed vesicles. From that and from the previously mentioned fact that final vesicles are of similar size with seed vesicles, it can be deducted that added amphiphilic molecules incorporate into vesicle membranes and cause vesicles’ growth and fission.
2.2 No leakage during fission

The study of Markvoort and Pfleger\textsuperscript{10} showed that vesicles do not leak during fission. They used a fluorescence dye and enclosed it into vesicles. At first fluorescence signal was very low. It was probably caused by the binding of some dye to the outside of the vesicles (Fig. 3). At time point 2 (shown in Fig. 3), fatty acids were added at pH 10.5 (at higher pH self-assembly of amphiphilic molecules is more favourable). Fluorescence increased slightly, but from a control experiment the rise was ascribed only to pH increase. At time point 3, a substance known to destroy vesicle membranes was added. The result was a great increase of fluorescence, because the membranes were torn and the dye was now not hidden inside the vesicles anymore. Via dynamic light scattering a control experiment was performed, showing that the enclosed dye does not affect the process of formation of new vesicles. This way, they could be sure, that vesicle fission did occur between time points 2 and 3 and that there was no leakage as the fluorescence did not increase.

![Figure 3](image)

\textbf{Figure 3:} This graph shows relative intensity of fluorescence measured in water solution. At time point 1 vesicles containing fluorescent dye were present in water solution. At time point 2 micelles at pH 10.5 were added. Change of pH induced an increase in intensity of fluorescence. At time point 3 TX-100 was added. It is a substance that tears apart vesicle membranes. The signal drops to zero occurred when the sample was removed for micelles and TX-100 to be added.\textsuperscript{10}

There are several hypothetical mechanisms for vesicle fission: fission by budding,\textsuperscript{10, 12, 7} by internal vesicle assembly and then birthing\textsuperscript{13, 11} or by elongating into thread-like form and then dividing into several vesicles.\textsuperscript{11}

2.3 Fission by budding

Budding is a process where a vesicle changes its shape into a pear-like form. This is because at given ratio between vesicle area and vesicle volume a pear-like shape is stable whereas the spherical shape is not stable anymore. The vesicle then continues changing through stable states towards a form of two spheres, connected by a neck (Fig. 4).\textsuperscript{12, 7} When a neck breaks we get two spherical vesicles. Budding is driven by osmotically induced changes of a ratio between surface area and vesicle volume or by a difference between outer and inner leaflet
area of a membrane. This difference can be caused thermally, by an asymmetry of the membrane or simply by such a high concentration of amphiphilic molecules added into the water solution that they incorporate into the outer monolayer of a vesicle much faster than they can flip to the inner monolayer.

In a study of Markvoort and Pfleger it was shown that the difference between monolayer areas can be a driving force for budding and fission. The experiment consisted of three parts. The first one was the already mentioned matrix effect experiment that showed creation of new vesicles. Secondly they decreased the rate of adding fatty acids into water solution, so that the excess material had time to flip into the inner layer. The results after 10 hours of slow adding of fatty acids showed that vesicles grew but fission did not take place. The third part was a coarse-grained molecular-dynamics simulation of a vesicle in water being added fatty acids (Fig. 5). In simulation they used three different particles: head particle, tail particle and water particle. Fatty acids were represented by a chain of two head particles and four tail particles. Water particles represented four molecules of water each. The forces between particles were specified. The result of the simulation was vesicle fission with no leakage.

**Figure 4**: Vesicle budding: spherical vesicle grows into an elongated shape, a bud connected to the vesicle by the neck is formed, the neck breaks and there are two vesicles instead of one.

**Figure 5**: Simulation of vesicle fission by budding. In panel A are presented coarse-grained simulations of fatty acid and water particles that were used. Fatty acids forming initial vesicles are coloured grey, freshly added ones are coloured red, as seen in panel B. Water particles are not shown. Panel C shows the initial state: already elongated vesicle and added fatty acids that later (in panel D) incorporate directly into the vesicle or form micelles, that eventually also merge with the vesicle. In panel E a neck and a bud are formed and in panel F the neck had been broken and there are two vesicles.
In this simulation the initial form of the vesicle was ellipsoidal and the vesicle was quite small. Had they chosen a spherical vesicle, it would have to grow first and its area to volume ratio would have to change, only then to become ellipsoidal, then budded and in the end to break into two vesicles. So by choosing an ellipsoidal initial form they saved computational time, because their interest was in the division and not in the growth of the vesicle.  

This simulation showed that fission of a vesicle can happen by budding. Here it is important to note that membranes of studied vesicles consisted of fatty acids, which were also added in water solution. The results cannot in general be valid for every vesicle, as some are made of different and more complex amphiphilic molecules. Fatty acids are the simplest of amphiphilic molecules. They consist of a hydrocarbon chain (hydrophobic tail) and a carboxylic group at the end (hydrophilic head).

### 2.3.1 Selectivity mechanism

In their article Božič and Svetina gave a condition for a vesicle to reach a budded shape, which is necessary for a vesicle to divide in a previously discussed way. Unlike the previous experiments, where molecule properties defined vesicle behaviour, here a macroscopic description is used, where membrane is ascribed elastic properties and hydraulic permeability. The spontaneous curvature model is used. In general the elastic energy is a sum of stretching, shear and bending energy terms, but since a lipid bilayer is a 2D liquid, it does not exhibit shear elasticity. The stretching energy term is negligible so in this model elastic energy reads

\[
W_{el} = W_b + W_{bg} = \frac{1}{2} \kappa \int (C_1 + C_2 - C_0)^2 dA + \kappa_G \int C_1 C_2 dA ,
\]

where \( W_b \) is the bending energy, \( W_{bg} \) is the Gaussian bending energy, \( \kappa \) is the membrane bending modulus, \( \kappa_G \) is the saddle-splay modulus, \( C_1 \) and \( C_2 \) are the principal curvatures and \( C_0 \) is the spontaneous curvature. The integrals are being integrated over the membrane area \( A \). The spontaneous curvature \( C_0 \) is a material property, measuring the asymmetry of a membrane. For a closed surface of genus \( n \), \( W_{bg} \) contributes a constant value of \( 4\pi n \kappa_G \) to the membrane energy. So it has to be considered only when the vesicle topology changes.

For the division to take place the ratio between vesicle area \( A \) and vesicle volume \( V \) must increase, so that the vesicle becomes flaccid. When it does, the membrane starts to fold and a bud is created, which leads into vesicle fission.

Vesicle area \( A \) in time changes as

\[
\frac{dA}{dt} = \frac{\ln 2}{T_d} A .
\]

\( T_d \) is the time in which vesicle area doubles. Volume of a vesicle changes in time because the membrane is permeable. The rate of this change is determined by pressure difference between
outside and inside of the vesicle $\Delta p$, by vesicle area $A$ and by membrane hydraulic permeability $L_p$, which is constant in the used model:

$$\frac{dV}{dt} = L_p A \Delta p.$$  \hspace{1cm} (3)

From the minimization of $W_b$ it follows that vesicle shapes depend only on the reduced spontaneous curvature $c_0$ and reduced volume $v$:

$$c_0 = C_0 R, \quad v = \frac{6 V \sqrt{\pi}}{A^{3/2}}.$$  \hspace{1cm} (4)

Here $R$ is radius of a sphere with area $A$ and $v$ is a ratio between volume of a vesicle and volume of a sphere with area $A$.

Vesicle shape behaviour can be represented in a $c_0(v)$ phase diagram (Fig. 6a). Lines $L^{pear}$, $D^{pear}$ and $\Delta p = 0$ show borders between shapes of different symmetries. For example on the $L^{pear}$ line lie the budded shapes of two spheres connected by an infinitesimally narrow neck.

Evolution of the system is described by

$$\frac{1}{\ln 2} \frac{dv}{d\tau} = -\frac{3}{2} v - \frac{18 \eta}{C_0^4} \frac{\partial w_b(c_0, v)}{\partial v}$$  \hspace{1cm} (5)

where $\tau = \frac{t}{T_d}$ is reduced time, $w_b(c_0, v) = \frac{W_b(c_0, v)}{8\pi \kappa}$ is the reduced bending energy whose derivative with respect to $v$ is known, as it is an inherent property of the used model and $\eta = T_d L_p \kappa C_0^4$ is the only parameter on which the behaviour of a system depends. Doubling time $T_d$ can be changed during an experiment by the change of the concentration of added amphiphiles, whereas $L_p$, $C_0$ and $\kappa$ are properties determined by vesicle constituents and
cannot be changed during an experiment. Their values can only be altered if another experiment with vesicles made of other constituents is performed.

Breaking of a neck is energetically favourable because of the release of the bending energy. The neck is an extremely curved membrane region therefore its energy corresponds to the change of Gaussian bending energy, which is given by $4\pi K_G$. It can be assumed that when a vesicle is transformed into the shape of two spheres connected by a very thin neck, it will divide. To set a condition for vesicle self-reproduction it only remains to set conditions for a vesicle to be able to grow from a spherical into a budded shape. In a $c_0(\nu)$ phase diagram budded shapes lie on a $L^{pear}$ line, which starts at $c_0 = 2\sqrt{2}, \nu = 1/\sqrt{2}$. If $\eta$ is too small, vesicle does not reach the boundary value for $c_0$, which means it does not become budded. Such vesicles are expected to grow into an elongated shape and never to divide. Only if $\eta \geq 1.85$ does a line, describing vesicle’s $c_0$ as a function of $\nu$, hit the $L^{pear}$ line and a vesicle undergoes fission.

### 2.3.2 Fission of myelin-like giant multilamellar vesicles

Budding is also the way of fission of myelin-like giant multilamellar vesicles (mGMVs) as shown by Takakura and Sugawara. The mGMVs are giant multilamellar vesicles with tightly packed bilayer membranes (Fig. 7a). This enables a cooperative interlamellar reorientation of amphiphiles. Vesicles in this experiment consisted of amphiphiles with two hydrophobic tails. They were added bolaamphiphilic molecules. These are molecules that consist of two hydrophilic parts separated by a hydrophobic part. In water they hydrolyse into an electrolyte and an amphiphile, in this case equal to the amphiphiles that build the vesicle. After the addition, vesicles started to grow and divide. The division is a consequence of a disturbance of the membrane caused by a change in osmotic pressure or by the effect of an electrolyte. Because of the closeness of the bilayers, the destabilization of the outer bilayer induces reorientation of molecules of other bilayers. This deformation then leads into budding and fission (Fig. 7b). Products of the fission are both mGMVs that can grow and divide again if they are added the bolaamphiphiles.

![Figure 7](image.png)

**Figure 7:** a) Comparison of a unilamellar and a multilamellar vesicle. b) Fission of an mGMV happens via budding. Images are taken 0, 1.5, 3.5, 4, 5, 10 and 50 minutes, respectively, after the addition of bolaamphiphiles. The white bars correspond to 10 µm.
In a separate experiment only electrolyte was added. In this case vesicles divided without growing. That is why an electrolyte was suggested to act as a trigger of the division of mGMVs.  

2.4 Other mechanisms of fission

Another possible pathway for vesicle fission is internal synthesis of new vesicles, which are afterwards released from the mother vesicle. Internal synthesis can happen in two ways. New amphiphiles can be enzymatically synthesised inside a vesicle, or mineral surfaces may be encapsulated inside a vesicle and accelerate new vesicle assembly from fatty acid micelles. When inner vesicles grow to a certain size, they can be released from the mother vesicle without rupture of its membrane. This process is called birthing (Fig. 8). It was microscopically observed but its mechanism remains unclear.

![Figure 8](image)

**Figure 8:** A schematic representation of self-reproduction vesicle pathways. Pathway (a) shows birthing. An inclusion vesicle is formed and then expelled from the mother vesicle. Pathway (b) shows vesicle fission via budding.

Vesicle fission by birthing can happen in another way, similar to fission by budding. In this case instead of changing from spherical to budded shape, it changes from spherical to stomatocyte form. Membrane invaginates and forms a cavity connected with the outside by a thin neck. Inclusion vesicle is then formed by pinching of the invagination neck and after that expelled by birthing. It depends on membrane properties whether a vesicle will follow the path of budding or of invagination.

Sakuma and Imai showed that for a vesicle consisting of two sorts of lipids: inverse-cone-shaped DLPE (1, 2-dilauroyl-sn-glycero-3-phosphoethanolamine) and cylinder-shaped DPPC (1, 2-dipalmitoyl-sn-glycero-3-phosphocholine), the path of vesicle fission depends on the molar fraction \( \phi \) of DLPE:

\[
\phi = \frac{n_{DLPE}}{n},
\]

where \( n_{DLPE} \) is the number of DLPE vesicles and \( n \) is the total number of all the lipids in a vesicle.
In their experiment vesicle fission was induced with a temperature change. Vesicles were not added any amphiphilic molecules for a membrane growth, the ratio between area $A$ and volume $V$ increased only because of the temperature change. Temperature was changed from 35°C where vesicles were spherical to 42°C where chain melting of DPPC took place. Then the temperature was decreased back to 35°C.  

Vesicles with $\phi < 15\%$ deformed into a stomatocyte form when temperature increased and returned into a spherical shape after temperature decrease (Fig. 9). Vesicles with $15\% \leq \phi < 30\%$ followed the budding path. A bud was formed, the neck broke and daughter vesicles were formed. After decrease of temperature daughter and mother vesicles recovered a spherical form. Vesicles with $30\% \leq \phi < 45\%$ deformed into a stomatocyte form and then formed inclusion vesicles after the breaking of the necks. When the temperature was lowered, the area of the mother vesicle decreased which resulted in increased pressure inside. This caused the formation of a pore in a membrane of a mother vesicle, through which daughter vesicles were expelled. Afterwards the pore closed. For vesicles with $\phi > 50\%$ no production of daughter vesicles was observed. Vesicles deformed into a stomatocyte or into a budded shape depending on their DLPE molar ratio. The deformation was driven by the increase of the ratio between vesicle area and vesicle. In fluorescence images no spatial separation (no formation of domains) of DLPE and DPPC molecules was observed as a possible cause for vesicle deformation.

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**Figure 9:** Different paths of vesicle fission are followed for different molar fractions of DLPE lipids in the vesicle membranes. Budding occurs for $15\% < \phi < 30\%$ and internal vesicle assembly followed by birthing for $\phi > 30\%$. The scale bar is 10 μm.
In 2009 Zhu and Szostak\textsuperscript{16} published an article describing yet another path of vesicle division. In their experiment they used large multilamellar vesicles (\(\sim 4 \mu\text{m}\) in diameter). By addition of amphiphiles and micelles, they incorporated into the outer membrane much faster than water could pass through it and increase the volume between bilayers. The membrane had to deform in a way that required no additional volume, and therefore a thin tail or two were formed. Tails then elongated and vesicles shrank until they became completely thread-like (Fig. 10). These vesicles divided into several daughter vesicles with no losing of cargo, when disturbed by mild shear forces. The forces were realised by blowing air into the solution. In this way it is possible to create a great number of vesicles at the time and it also seems a very good solution for creation of artificial life, because it is very robust and no content is spilled at fission.\textsuperscript{16}

\textbf{Figure 10:} In multilamellar vesicles a thin tail (or two) emerges after addition of amphiphilic molecules and micelles. The spherical part of the vesicle then shrinks and the tail elongates until all there is is a long thread-like vesicle. Under small agitation this long vesicle divides into several spherical vesicles which are multilamellar themselves.\textsuperscript{16}

Vesicles are very important in view of artificial life, because they represent closed compartments that are necessary for life to begin.\textsuperscript{9} Because of their ability to self-reproduce, they are regarded as very probable predecessors of the first living cells. But they are not alive, as they cannot store information in order to undergo Darwinian evolution.

\section{3 Synthesising life}

Previously various ways of vesicle self-reproduction were discussed. For synthetic life however, vesicle production and fission are not enough. Storing information and catalysing metabolism are also very important.\textsuperscript{3} There is much and more to say about these two topics and about synthesising life in general but in this seminar we will only shortly present a possible way of realising the synthesis of life.

There are several theories explaining how compartments and information storage could have been realised in early life: RNA in a lipid vesicle,\textsuperscript{3} RNA in a protein made compartment,\textsuperscript{17} lipids as carriers of information inside a lipid vesicle,\textsuperscript{17} and so on.
In order to synthesise an artificial cell, the former seems most promising. It is also most similar to today’s cells. The ‘RNA world’ hypothesis suggests that primordial cells used RNA as storage of genetic information and as enzymes that catalyze metabolism, because they were lacking protein synthesis. Because RNA is able to do both and because in today’s cells RNA and DNA are the only currently used genetic materials, RNA becomes the most obvious choice for the making of artificial cell. It is simpler than DNA and stable enough.

Experimentalists are trying to evolve or design an RNA replicase. This is an RNA molecule that is able to act as a template for storage and transmission of genetic information and as an RNA polymerase that can replicate its own sequence. When (or if) they succeed, such RNA replicase will probably be a key feature of artificial cells. However, such a molecule is not alive by itself. It cannot replicate itself, so there must be a second such molecule nearby. One must act as a polymerase and the other one as a template. To achieve this, concentration of RNA replicase must be extremely high, or RNA replicases must be confined in a closed compartment. The later is preferable, for in compartments advantageous mutations can cause more efficient replicating and therefore these compartments have an advantage over compartments in which advantageous mutations did not take place.

For a structure in which replicating RNA is confined, lipid vesicles can be used, as we showed that they are able to self-reproduce. Still, there is something missing: a coupling between the compartment and its cargo. Vesicles and RNA replicase must replicate at a similar rate and the replications must be connected. This could be achieved by a ribozyme, capable of synthesis of amphiphilic molecules. Ribozyme is short for ribonucleic acid enzyme. It is an RNA molecule with the ability to catalyze chemical reactions. Growth and fission of membranes ‘at will’ would be enabled by synthesis of amphiphiles. With this coupling, vesicle with its RNA cargo could be considered an organism, able to evolve as a whole.

This is a theory, suggesting a way of creating artificial cellular life. Synthetic life has not been realised as yet. Whether it will ever be realised in this way, or some other method will work better, cannot be forecast at this stage.

Unlike artificial cells artificial organelles have been synthesised. Approaches to construct an artificial cell and an artificial organelle are somewhat different. Organelles are designed to perform specific tasks, for example delivery of drugs, enzymes, nucleotides and diagnostic agents. They can be produced in a top-down approach. Information system (RNA) can be reduced to perform these tasks and put into a closed compartment. Membranes must be stable enough and they must be designed in a way that enables organelles to target the right cells. This is different than in artificial cells, because in this case the membranes must be able to self-reproduce. Because of their biochemical capabilities, artificial organelles are suited for biomedical application and this is a path that their development follows.

Artificial organelles are not strictly speaking alive as they cannot self-reproduce so they are not exactly in the domain of artificial life. They are mentioned here because they are an important part of synthetic biology and because of their importance in biomedicine.
4 Conclusion

The main preoccupation of scientists, working on biochemical-based artificial life, is to synthesise a living minimal cell from scratch. A living cell here refers to an entity that can self-reproduce and is subject to Darwinian evolution. This is why it must contain some kind of information storage. RNA molecules were proposed for this function as they perform it in some today’s cells as well. This RNA would have to be able to store information and act as a template for transmission of genetic information. Because RNA is also capable of acting as an enzyme, to keep a cell as simple as possible, RNA would have to catalyze metabolism too. For containment of information storage a cell must be confined inside a close compartment. Vesicles seem quite suited to do the job. They are able to self-reproduce in different ways, provided they meet certain requirements.

Many paths have been proposed for synthesis of a minimal cell and many studies are aiming in this direction, but until now no artificial cell was synthesised.

References