CONSTRUCTING PROTOCELLS: A SECOND ORIGIN OF LIFE

STEEN RASMUSSEN
Center for Fundamental Living Technology (FLinT)
Santa Fe Institute, New Mexico USA

ANDERS ALBERTSEN, PERNILLE LYKKE PEDERSEN, CARSTEN SVANEBORG
Center for Fundamental Living Technology (FLinT)

ABSTRACT: What is life? How does nonliving materials become alive? How did the first living cells emerge on Earth? How can artificial living processes be useful for technology? These are the kinds of questions we seek to address by assembling minimal living systems from scratch.

Introduction

To create living materials from nonliving materials, we first need to understand what life is. Today life is believed to be a physical process, where the properties of life emerge from the dynamics of material interactions. This has not always been the assumption, as living matter at least since the origin of Hindu medicine some 5000 years ago, was believed to have a metaphysical vital force.

Von Neumann, the inventor of the modern computer, realized that if life is a physical process it should be possible to implement life in other media than biochemistry. In the 1950s, he was one of the first to propose the possibilities of implementing living processes in computers and robots. This perspective, while being controversial, is gaining momentum in many scientific communities.

There is not a generally agreed upon definition of life within the scientific community, as there is a grey zone of interesting processes between nonliving and living matter. Our work on assembling minimal physicochemical life is based on three criteria, which most biological life forms satisfy. For a comprehensive discussion of minimal cells, see Rasmussen *et al.,* 2009 and for a snapshot of the broader field of Artificial Life see e.g. Fellermann *et al.,* 2010. From an operational point of view, a minimal living physicochemical system needs to:

(1) use free energy to convert resources from the environment into building blocks so that it can grow and eventually divide.
(2) have the growth and division processes at least partly controlled by inheritable information.
(3) allow the inheritable information to change slightly from one generation to the next, thereby permitting variation of the growth and division processes and thus allowing selection and hence evolution.

However, these criteria are not quite adequate. Think for example of a mule, a cross between a horse and a donkey. The mule is sterile and can neither procreate nor undergo evolution. Similarly, most ants in a colony are also unable to procreate. Still, most people believe that both a mule and an ant are alive. Influenza viruses have no metabolism and they undergo vivid evolution. But due to the lack of metabolism a virus is usually considered not to be alive. A flame from a match has a metabolism, as it converts chemical energy into heat, it can grow and multiply, but it cannot undergo evolution. Therefore a flame is not alive according to the above criteria. Confused? It does not get easier when we think about robots and computers. Today there are many implementations of computer network processes, with self-replicating and evolving algorithms, which according to the above criteria should be regarded as living [Adami, 1998]. We have not yet created robots we would call alive according to the above criteria, but work is underway particular within the area of self-reconfigurable modular robots [Wiki, 2011].

It should be emphasized that much more effort within synthetic biology today [Porcar *et al.,* 2011] is devoted to modify existing living organisms than to create minimal living cells from scratch. The approach based on modifying existing cells is called the «top down» approach. The «bottom up» approach to creating minimal living cells can be pursued either by assembling existing biological building blocks in simplified ways or by only using non-biological building blocks, see Figure 1. The top down approach reached an important milestone

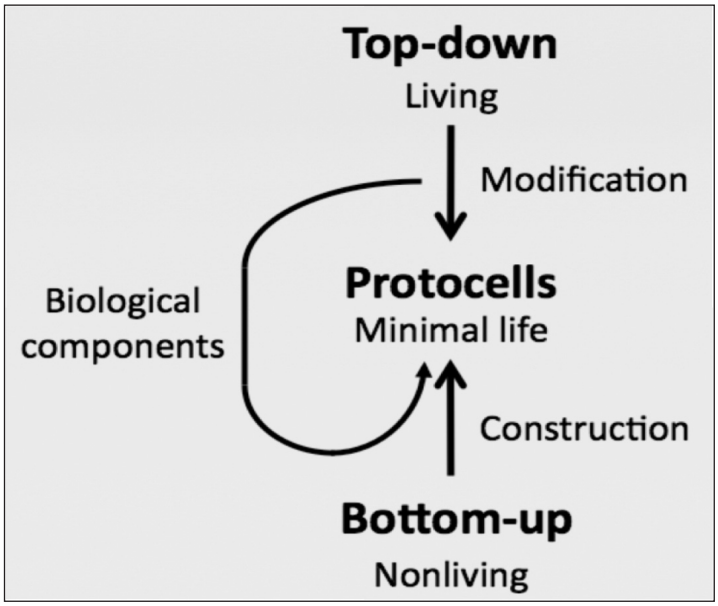


FIGURE 1: *The bottom up and the top down apaches in synthetic biology. We belong to the bottom up tradition.*

in 2010, when Craig Venter's team was able to transplant an artificially synthesized genome into another cell without a genome and thereby «reboot» the other cell and bring it back to life [Gibson *et al.,* 2010] [Rasmussen, 2010]. Another important line of research within the top down tradition is the effort to develop so-called «bio-bricks» [BioBricks, 2011] that can be composed and inserted into cells, in similar ways as electrical engineers make and compose electronic components in modern information and communication technology devices. This would enable these modified cells to obtain novel useful properties such the ability to produce biofuels or pharmaceuticals.

The bottom up approach is pursued in the spirit of Richard Feymann [1] «What I cannot create, I do not understand». Our work belongs to the bottom up approach. Further, none of our molecules are found in modern cells but they all have similar functionalities. We use alternative, simpler and specially designed molecules because they allow us to realize the same fundamental functionalities using a dramatically simpler blueprint for the protocell compared to what we see in modern cells.

Minimal protocell design

Modern biological cells, as we know them from life on Earth today, are the result of several billion years of evolution. A modern cell consists of many complex cellular components where a myriad of reactions and processes take place, all of which are controlled by many different molecules. Some organisms consist of several trillion cells working together while others consist of only a single cell. The protocell that we are assembling from the bottom up is very different and much simpler than modern cells [2]. It consists only of three components, inspired by the most critical parts of modern cells: An information system («genes»), an energy transduction system («metabolism») and a container («cell body»), see Figure 2 and Rasmussen *et al.,* 2003 & 2004.

Protocell containers

We work with several different types of containers: Oil droplets, vesicles and reverse micelles, see Figure 3. Common to all of them is that their boundary is composed of simple fatty acids. In modern cells, the cell membrane structure is made of the much more complex phospholipids. Fatty acids and phospholipids

[1] On his blackboard at time of death in 1988; as quoted in *The Universe in a Nutshell* by Stephan Hawking. Richard Feymann (1918-1988) was a physicist and Nobel Prize Winner.

[2] The initial idea behind our protocell design and the initial work on the protocell implementation were developed at Los Alamos National Laboratory, see http://protocells.lanl.gov.

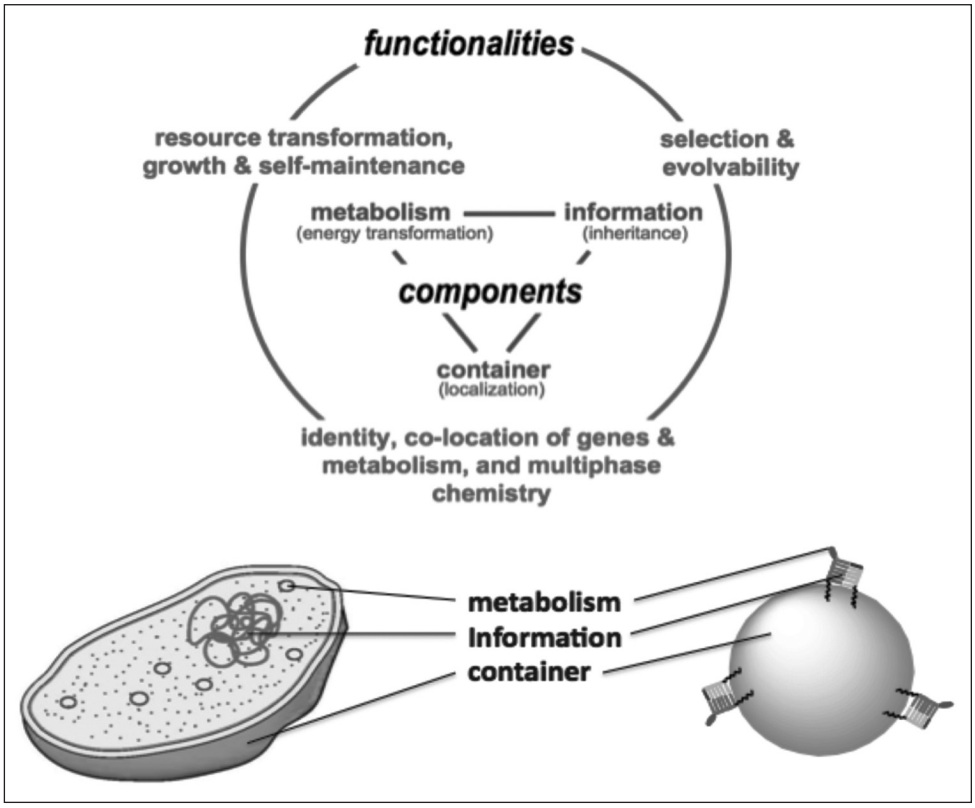


FIGURE 2: *(Top) The central protocell components: information, metabolism, and container and their functionalities. (Bottom) The protocell (right) compared with a modern bacterial cell (left). The cells are not to scale as the protocell is much smaller than the modern cell.*

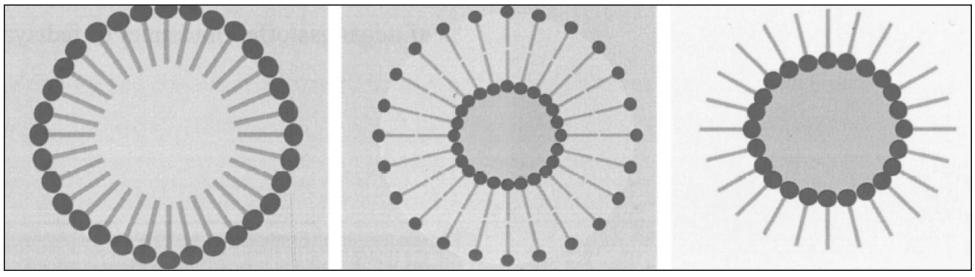


FIGURE 3: *(1) Oil droplet in water, which comprises a single layer of amphiphilic molecules, where the hydrophobic parts are facing inward toward the oil. (2) Vesicle consisting of a bilayer of fatty acids in which the hydrophobic parts are facing each other and the hydrophilic parts are facing the water. (3) Reverse micelle, which consists of a single layer of fatty acids, where the hydrophobic parts are facing outwards towards an organic solvent.*

are called amphiphilic molecules because they contain a hydrophobic- and hydrophilic part, and they will under the right conditions self-assemble into various container structures. The reason why we do not use phospholipids to build the protocell container is that their synthesis is far more complex and it would result in an overly complex metabolic system to create them from scratch. Moreover, we find traces of fatty acids in a number of meteors, making it likely that fatty acids were present on the early Earth.

In modern cells, all cellular components are found inside the cell. However, for our protocell design, both the information- and metabolic components use an anchor that enables them to attach to the surface of the container. This makes access to resources and disposal of waste much easier, as it can occur directly into the surrounding media and does not need to pass through a membrane. The anchor is composed of a long aliphatic chain, e.g. the hydrophobic portion of the fatty acid, that inserts itself between the fatty acids constituting the container, and thereby tethers the information- and metabolic components to the container. The protocell container can be thought of as a piece of used and sticky chewing gum that you decorate with information- and metabolism molecules. Vesicles are similar to modern cells in the container structure, as modern cells also have a bilayer membrane, but both the information- and the metabolism systems are on the «inside» of the membrane in a modern cell. Our reverse micelle based protocells resemble modern cells in the sense that they also have their metabolism- and information systems in the water cavity. However, the reverse micelles do not exist in an aqueous solution, but require an organic solvent (e.g. in a mixture of isooctane and octanol) to form.

Protocell metabolism and information systems

The metabolism in the protocell consists of a photochemical reaction that transforms an oily feedstock (a picolinium ester) from the environment into a fatty acid (decanoic acid). The metabolic complex that converts light energy into chemical energy in our system is a ruthenium complex. In the photochemical reaction, the «genetic material», in the form of a modified nucleobase (8-oxo-guanine) within a DNA sequence, catalyzes (controls) the production of container molecules. The catalytic efficiency depends on the DNA sequence, the 8-oxo-guanine amount and the proximity to the metabolic ruthenium complex. This information controlled metabolic production of container molecules has been realized in the laboratory [DeClue *et al.,* 2009; Maurer *et al.,* 2011]. With this simple metabolic mechanism, there is no need for the complicated modern translation machinery where DNA is translated into proteins that perform most functions in the modern cell, including the control of the metabolism. The informational molecule directly controls the metabolism without any intermediate step that requires proteins. Despite these significant differences, the protocellular metabolism is driven by light energy just as modern metabolisms in plants, algae and cyanobacteria.

As the protocellular metabolism slowly converts the oily feedstock into fatty acid, the container grows and under certain conditions it becomes unstable and divides into two or more smaller containers. The container fission can also be induced artificially by extrusion of the protocell through a filter. Both these processes are realized in the laboratory. However, before the container division occurs the modified DNA anchored to the container has to be copied using small resource DNA fragments from the environment. These precursor DNAs have to be digested and activated by the metabolism, in a similar way as the oily container resource molecules are converted to functional fatty acids, before they can be used as DNA building blocks. This container-associated replication of the modified DNA has yet to be realized in the laboratory.

During the DNA replication errors (mutations) may occur. This either results in genetic material that is either better or worse suited for catalyzing the photochemical reaction and thus it creates the possibility for selection and eventually evolution. This process has not yet been realized in the laboratory.

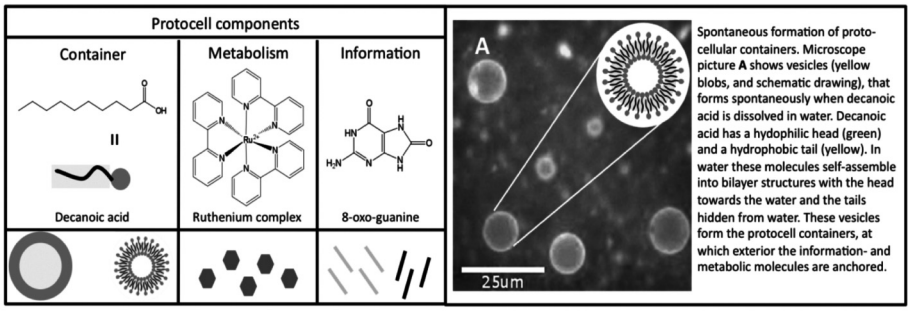
Protocell life-cycles

Life is a process, which is why protocells are not static entities. They undergo a cyclic process consisting of feeding, information replication, growth and division. However, it can be very difficult to predict what will happen when complex chemical reactions play together, which is necessary to develop such a protocellular life-cycle. Therefore, we use computer simulations of the various chemical reactions to calculate the possible ways in which the protocell's components can interact.

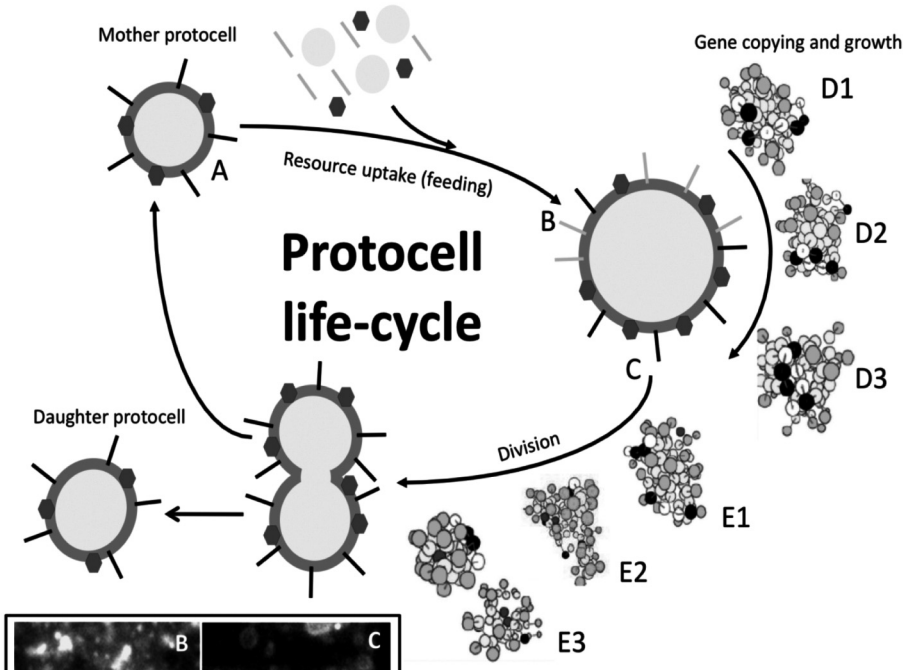
Figure 4 shows a protocellular life-cycle and the molecules it is composed of. The figure summarizes the self-assembly of protocells (A), feeding (B) and the result of the light driven and information controlled metabolism (C). We also show a computer simulation of two of the critical steps in the protocellular life-cycle: replication of the information molecule (E1-E3) and metabolic driven protocellular container division (D1-D3). This simple protocell design has made it possible to demonstrate experimentally how primitive information, metabolism and container can be coupled and function as a unit. Although most of the steps in the lifecycle already are demonstrated in the laboratory, the full life-cycle is not yet complete.

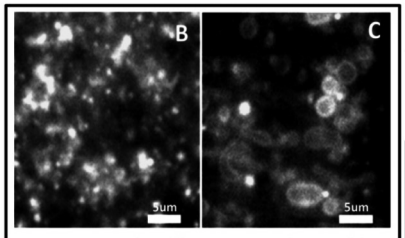
It may be useful to summarize the main differences between these protocells and modern cells:

(1) Our protocells are much simpler than modern cells.
(2) None of the protocellular molecules are found in modern biological cells.
(3) Both the protocellular information- and metabolic systems are attached to the outside of the container rather than being inside the cell. This means that it is not necessary to have proteins or other complex molecules to transport nutrients and waste through the cell membrane.



Spontaneous formation of protocellular containers. Microscope picture **A** shows vesicles (yellow blobs, and schematic drawing), that forms spontaneously when decanoic acid is dissolved in water. Decanoic acid has a hydophilic head (green) and a hydrophobic tail (yellow). In water these molecules self-assemble into bilayer structures with the head towards the water and the tails hidden from water. These vesicles form the protocell containers, at which exterior the information- and metabolic molecules are anchored.





Microscope pictures of two different processes in the protocellular growth phase. In **B** a population of protocells is seen after the feeding of decanoic acid vesicles with piculinium ester, but before starting the light driven metabolic process. The oily piculinum ester is seen to accumulate in the vesicles (strong yellow florescence) as they swell up. **C** shows the same population after the metabolic process, where the oily precursors are converted into surfactants (decanoic acid). The vesicles clearly grows and and change shape. The metabolic process is controlled by 8-oxo-guanine combined with the photoactive ruthenium complex, which converts light energy into chemical energy producing new container molecules that enlarges the vesicles.

Computer simulations of information molecular replication (**D1-3**) and container division (**E1-3**). All molecular groups are represented by small balls in this simulation. The protocell container consists of an oil droplet with oil molecules (two yellow balls) in the center and surfactant molecules (yellow and green) at the surface. The protocell has two short precursor information molecules with oily tails (black-white ball pair with yellow balls attached) and a longer information sequence (black-white-white-black sequence with yellow balls) (**D1**). The long and the two short DNA molecules find each other (**D2**), are ligated and forms a new complementary information sequence (white-black-black-white), which concludes the information replication. The container division (**E1-3**) is controlled by the information molecules combined with the metabolic complexes (red balls), which converts some of the oil molecules (yellow-yellow) into surfactants (green-yellow). The simulation show how this metabolic process eventually makes the droplet unstable (**E2**) so that it divides spontaneously into two new droplets (**E3**). In this process each daughter cell get an information molecule and after a new resource uptake (feeding) the process can be iterated. Simulations are taken from Fellermann et al., *Artificial Life* **13** (2007) 319.

(4) The «genetic material» is involved directly in the metabolic process, in which the composition of the information sequence and the position of particular bases control the protocellular growth process.

Also, it may be useful to discuss our choice of molecular components for the protocell. As our information sequence we use modified DNA (DNA with a lipophilic anchor) because DNA is the easiest and least expensive templating molecule to work with. We initially used PNA (peptide nucleic acid), because of its attractive dual composition of peptides and nucleic acids. However, PNA turned out to be too complicated to work with. Also, we don't use modified RNA because it is less robust than DNA. Ruthenium tris bipyridine is chosen as the metabolic complex because it is the most well characterized photoactive molecule. Decanoic acid is used as the building block for the vesicles because of its ability to form stable containers at room temperature. Each of the other components we use has a similar history.

In short, the choice of molecular components is based on (i) simplicity, (ii)basic functionality, (iii) ease of use, and (iv) costs. As a result of these criteria we ended up with a set of molecular building blocks that is not found in modern biology. We did not seek to create from non-biological building blocks. Having to engineer a protocell bottom up, it ended up this way.

Living technology

What are the likely implications of artificial living processes and how can artificial living processes be useful? Making living materials from nonliving materials and the implementation of living processes in other media both address and pose fundamental epistemological questions [Rasmussen, 1991]. However, the potential usefulness of novel engineered living processes stem from the tantalizing properties of life itself. Living systems are characterized by energy efficiency, sustainability, robustness, autonomy, learning, local intelligence, self-repair, adaptation, self-replication and evolution [Bedau *et al.*, 2010; Bedau *et al.*, 2010b]. Unfortunately, these are desirable properties current technology lacks and it creates a variety of problems for our society.

It is not our place to make predictions about how future technology could become more alive, but instead we can summarize a vision that part of our scientific community share. This vision is not yet science, but more akin to science fiction. First a little historical background: During the 19th century, the industrial revolution automated mass production in factories and a vast transportation infrastructure. In the latter part of the 20th century and the start of current century, the information technological revolution automated personal information processing in computers and the Internet. We believe the next major technological revolution will be based on an integration of information processing and material production. Living organisms combine these processes seamlessly and biological organisms are still

the only machines that can do this. To find out how they do this is in part why we seek to understand life.

One of our concrete visions about living technology is the construction of a personal fabricator (PF) as an analog to the personal computer (PC). To get an idea of what it might imply to have a PF at your tabletop in a generation or so, imagine an advanced 3D printer, which is able to control microbiological fabrication as you see it in a modern bread-baking machine. The PC and the Internet technology have enabled the individual to create and share information. Living technology has the potential to give the individual access to the design, sharing and production of complex objects in a simple and sustainable manner [SPLiT, 2010]. Again, the sustainable personal fabricator network is a vision and its implementation still relies on years of basic research and dedicated engineering at the interfaces between nanoscience, biotechnology, production technology and information & communication technology.

Some of the ongoing activities within the emerging Chembio-ICT area can be followed at the European Commission sponsored project web pages for PACE, ECCell, MATCHIT and COBRA [Chembio-ICT, 2004-2010]. Common to these projects is an investigation of how to create and utilize living processes in a variety of hybrid bio-chemical, computational and robotic systems. As our technology becomes more biological it also brings us a variety of new safety, environmental, and ethical challenges. These issues are addressed by the one of the research networks at the Initiative for Science, Society and Policy [ISSP, 2011].

Acknowledgements: The presented summaries about the protocell results stem from the collective work of the research group at the Center for Fundamental Living Technology (FLinT) and our collaborators, which are greatly acknowledged. We are also grateful for the financial support provided by the Danish National Research Foundation (Dansk Grundforskningsfond), the University of Southern Denmark and the European Commission through the Chembio-ICT program and the MATCHIT, ECCell and COBRA projects.

References

Adami, C., *Introduction to artificial life,* Springer Verlag, 1998.

Bedau, M.; McCaskill, J. S.; Packard, N., and Rasmussen, S., «Living technology: Exploiting life's principles in technology» (2010), *Artificial Life* **16**, 89-97.

Bedau, M.; Hansen, P. G.; Parke, E., and Rasmussen, S. (eds.), *Living Technology* 5 Questions, Automatic Press/VIP, 2010

BioBricks 2011, see http://biobricks.org

Chembio-ICT, see e.g. http://fp7-matchit.eu, http://www.cobra-project.eu or http://homepage.ruhr-uni-bochum.de/john.mccaskill/ECCell/

DeClue, M.; Monnard, P.-A.; Bailey, J.; Maurer, S.; Collins, G.; Ziock, H.; Rasmussen, S., and Boncella, J. (2009), «Nucleobase mediated, photocatalytic vesicle formation from ester precursor molecules», *JACS* **131**, 931-933.

FELLERMANN, H.; RASMUSSEN, S.; ZIOCK, H., and SOLE, R. (2007), «Life-cycle of a minimal protocell – A dissipative particle dynamics study», *Artificial Life* **13**, 319-345.

FELLERMANN, H.; DORR, M.; HANCZYC, M.; LAURSEN, L.; MAUER, S.; MERKLE, D.; MONNARD, P.-A.; STOY, K., and RASMUSSEN, S. (eds.), *Artificial Life XII, Proceedings of the Twelfth International Conference on the Synthesis and Simulation of Living Systems,* eds., MIT Press online proceedings, 2010.

GIBSON, D. G., *et al.* (2010), «Creation of a bacterial cell controlled by a chemically synthesized genome», *Science* doi: 10.1126/science.1190719

INITIATIVE FOR SCIENCE, SOCIETY AND POLICY (ISSP), see http://science-society-policy.org under living technology.

MAURER, S.; DECLUE, M.; ALBERTSEN, A.; KUIPER, D.; ZIOCK, H.; RASMUSSEN, S.; BONCELLA, J., and MONNARD, P.-A. (2011), «Interactions between catalysts and amphiphilic structures and the implications for a protocell model», *Chem Phys Chem* **12**, 828-835.

PORCAR, M.; DANCHIN, A.; LORENZO, V.; DOS SANTOS, V. A.; KRASNOGOR, N.; RASMUSSEN, S., and MOYA, A. (2011), «Ten grad challenges for synthetic life, to appear», in *Synthetic Biology*.

RASMUSSEN, S., *Aspects of Information, Life, Reality, and Physics, Artificial Life II, SFI Studies in the Sciences of Complexity,* Vol. X, Ed. C. Langton *et al.,* Addison-Wesley, 1991, 767.

RASMUSSEN, S.; CHEN, L.; NILSSON, and ABE, S., «Bridging nonliving and living matter», *Artificial Life* **9**, 269-316.

RASMUSSEN, S.; CHEN, L.; DEAMER, D.; KRAUKER, D. C.; PACKARD, N. H.; STADLER, P. F., and BEDAU, M. A., «Transitions from nonliving to living matte»r (2004), *Science* **303**, 963.

RASMUSSEN, S.; BEDAU, M. A.; CHEN, L.; DEAMER, D.; KRAUKER, D. C.; PACKARD, N. H., and STADLER, P. F., *Protocells: Bridging nonliving and living matter,* MIT Press, Cambridge, 2009.

RASMUSSEN, S., «Life after the synthetic cell – Bottom up will be telling more» (2010), *Nature*, 465422a, May 20.

SPLiT 2010, *Sustainable Personal Fabricator Network,* see http://www.ecltech.org/LTFlagship/. The SPLiT vision was developed and lead by N. Packard, J. McCaskill and S. Rasmussen.

SUNAMI, T.; CASCHERA, F.; MORITA, Y.; TOYOTA, T.; NISHIMURA, K.; MATSUURA, T.; SUZUKI, H.; HANCZYC, M. M., and YOMO, T. (2010), «Detection of Association and Fusion of Giant Vesicles Using a Fluorescence-Activated Cell Sorter», *Langmuir* **26**, 15098-15103.

WIKI (2011), *Wikipedia, Self-reconfigurable modular robots,* see http://en.wikipedia.org/wiki/Self-reconfiguring_modular_robot

STEEN RASMUSSEN

Center for Fundamental Living Technology (FLinT)
Department of Physics and Chemistry
University of Southern Denmark
Santa Fe Institute, New Mexico USA
http://www.sdu.dk/flint
steen@ifk.sdu.dk

ALBERTSEN, PEDERSEN and SVANEBORG

Center for Fundamental Living Technology (FLinT)
Department of Physics and Chemistry
University of Southern Denmark