Assembling Living Materials and Engineering Life-like Technologies

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ABSTRACT
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 possibility of implementing genuine living processes in computers and robots. This perspective, while still controversial, is rapidly gaining momentum in many science and engineering communities. Below, we summarize our recent activities to create artificial life from scratch in physicochemical systems. We also outline the nature of the grand science and engineering challenges faced as we seek to realize Von Neumann's vision: Integration of information processing and material production from the nano- to the macroscale in technical systems.

Categories and Subject Descriptors
J.3 Computer Applications; LIFE AND MEDICAL SCIENCES: Biology and genetics.

General Terms: Algorithms, Design

Keywords
Minimal protocells, self-reproducing robots, chembio-ICT, living technology, sustainable personal fabricator network.

1. INTRODUCTION
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 [1] and for a snapshot of the broader field of Artificial Life at two different times, see e.g. [2,3]. 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 more importantly reproduce.
(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.

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 as used are found in modern cells, but several have identical components and 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.

It should be emphasized that although our team has been predominantly occupied with the bottom-up approach to the creation of artificial living systems, much more effort within synthetic biology today[4] 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 (see e.g., [5]) or by only using non-biological building blocks, see Figure 1. The top-down approach reached an important milestone 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.[6,7,8] Another important line of research within the top down tradition is the effort to develop so-called "bio-bricks"[9] 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.

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[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.

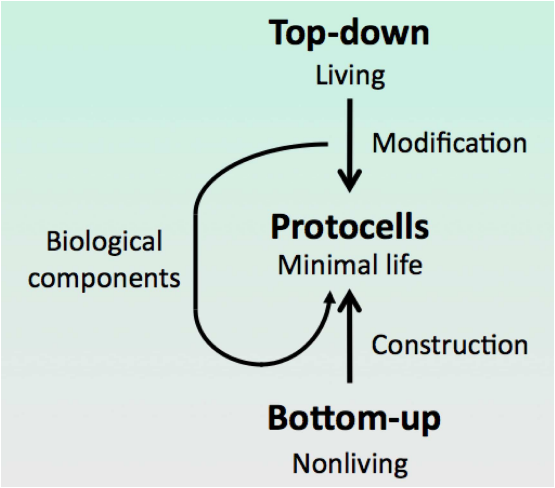

Figure 1. The bottom-up and the top-down approaches in synthetic biology. We belong to the bottom up tradition.

2. MINIMAL PROTOCELL DESIGN

Modern biological cells, as we know them from life on Earth today, are the result of several billion years of evolution. The latter part of this evolution generated more complex multicellular organisms in which the component cells underwent differentiation to take on specific roles. 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")[10,11,12], see Figure 2.

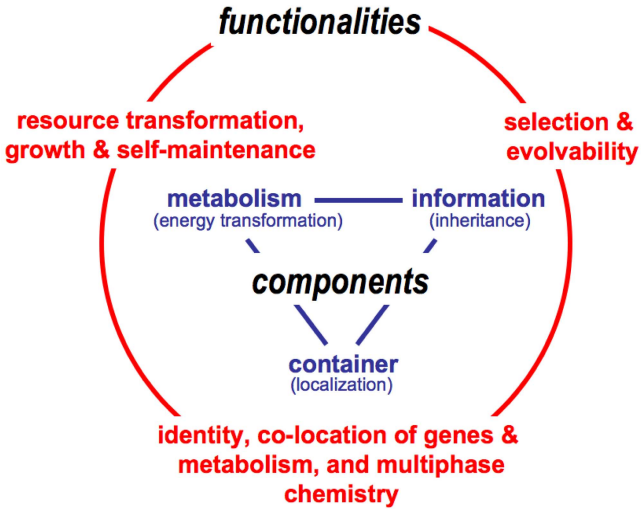

Figure 2. The central protocell components: information, metabolism, and container and their functionalities.

[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.

2.1 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 and contains complex transport and messaging subsystems. Fatty acids and phospholipids are called amphiphilic molecules because they contain a hydrophobic- and hydrophilic part, and under the right conditions they will 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.

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 from/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.

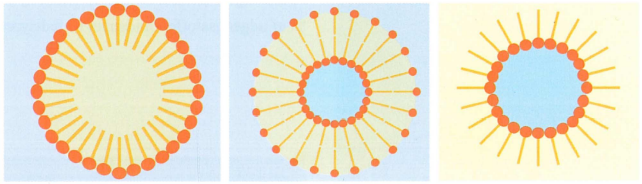

Figure 3 (1) Oil droplet in water, which comprises a single layer of amphiphilic molecules, where the hydrophobic parts are facing inward toward the oil. (2) Vesicle consisting of a bilayer of fatty acids in which the hydrophobic parts are facing each other and the hydrophilic parts are facing the water.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

2.2 Protocell metabolism and information systems

The metabolism in our 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 its

proximity to the metabolic ruthenium complex. This information controlled metabolic production of container molecules has been realized in the laboratory.[13,14] With this simple metabolic mechanism, there is no need for the complicated modern translation machinery where DNA is translated into proteins that perform most of the functions in the modern cells, including the control and implementation of the metabolism. In our case, the informational molecule directly controls the metabolism without any intermediate step that requires proteins. Despite these significant differences and the much more diverse set of compounds made in modern cells, the protocellular metabolism is driven by light energy just as photosynthesis is driven by light 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 DNA fragments have to be assembled in the proper order so as to be a copy of the parent DNA and chemically bonded into a contiguous strand by the metabolism. The metabolism employed here is essentially identical to that used to convert the oily container resource molecules are converted to functional fatty acids. A protecting group is cleaved from the DNA fragments to allow them to bond to their neighboring fragments that have aligned themselves in the proper order by base pairing with the parent DNA fragment. This replication process of the DNA is in the process of being realized in the laboratory.

During the DNA replication errors (mutations) may occur including improper base-pairing of slightly different DNA fragments or extension of the DNA caused by using fragments that partially overhang the end of the parent DNA. In this way both mutated and extended versions of the DNA are possible leading respectively to different information and potentially more information. This either results in genetic material that is either better or worse suited for catalyzing the photochemical reaction or possibly other reactions and thus it creates the possibility for selection and eventually evolution. This process has not yet been realized in the laboratory.

2.3 Protocell life-cycles

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 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 do not 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 primitive nature and 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 as a whole are not found in modern biology, even though we do make use of a modified DNA as the information molecule due to its ability to contain information and base-pair so that identical copies of it can be made. We did not seek to create from non-biological building blocks. Having to engineer a protocell bottom up, it ended up this way.

3. MINIMAL SELF-REPRODUCING ROBOTS

As for wet carbon chemistry, the field of robotics also has a corresponding grand challenge to assemble minimal macroscopic self-reproducing systems. The problem for the robotics community seems harder than the problem for the chemistry community mainly because the macroscopic world does not have self-assembly of its parts for free as the microscopic world has for appropriate molecular aggregates in water. Metabolism (energy) and information (algorithms) are equally important for robots and cells, while the container can be different in the two systems. For self-replicating robots a distributed architecture of energy harvesting, resource collecting, building block creating, as well as the assembly of the building block into new units may be more appropriate. The critical issue is organizational closure and not physical enclosure.

Simple examples of self-replicating robots are already developed and are based on the assembly of existing complex functional modules.[15] Lipson and others have suggested that this paradigm could be extended to self-replicating robots that autonomously assemble building blocks from a warehouse of parts with varying levels of complexity. Also the rapid development of 3D printing technology gives rise to new implementation possibilities, where a self-replicating robotic system could manufacture some of its own parts, while collecting others from a warehouse.[16] The GECCO community is set to spearhead exciting developments within this area in the coming years.[17,18]

4. LIVING TECHNOLOGY

What are the likely implications of artificial living processes and how can artificial living processes be made useful? Making living materials from nonliving materials and the implementation of living processes in other media both address and pose fundamental epistemological questions.[19] 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, and most importantly evolution through self-replication.[20,21] Unfortunately, these are desirable properties current technology lacks, which over the last centuries have created an increasing variety of problems for our societies.

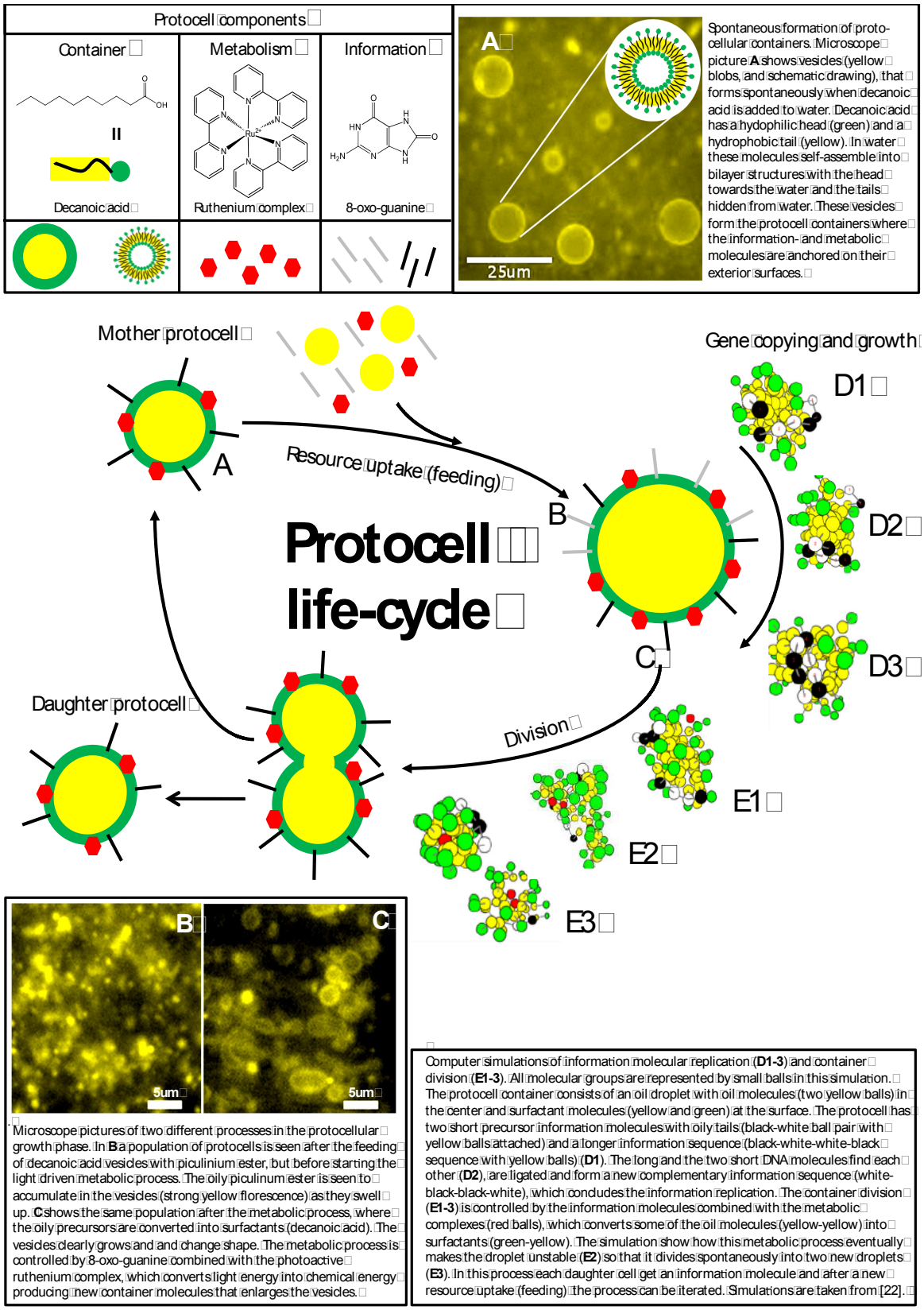

Figure 4. Protocell molecules, components and life-cycle.

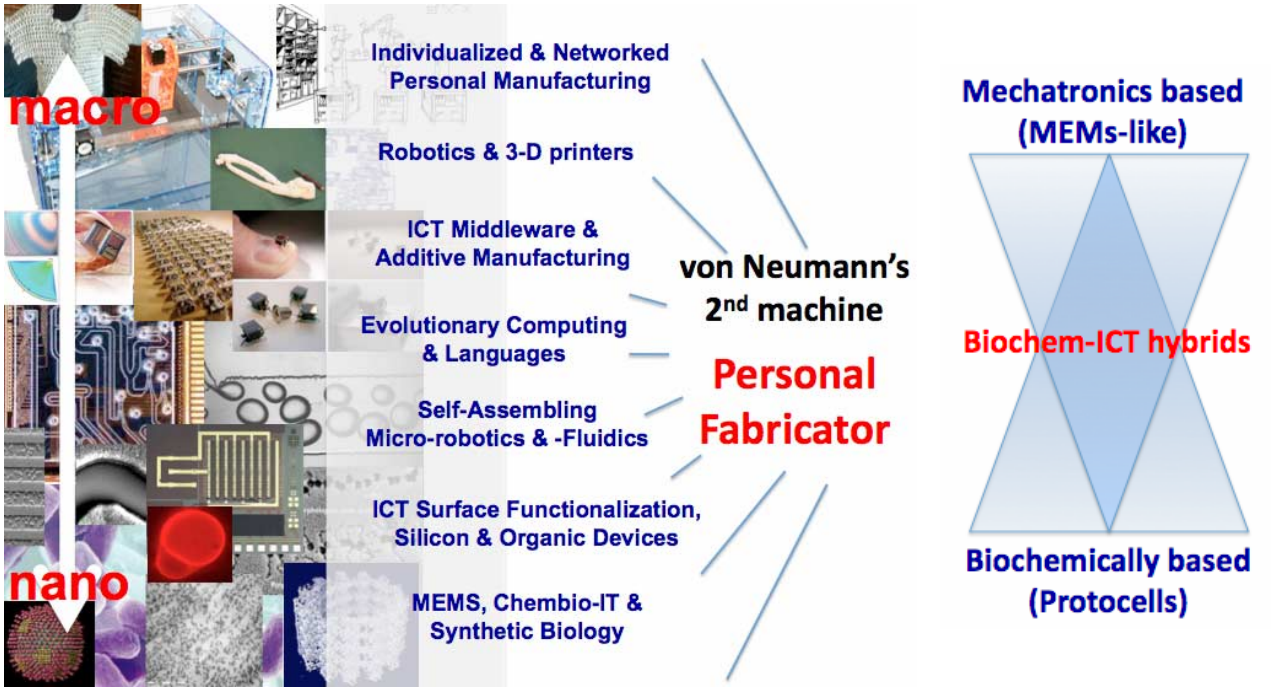

Figure 5 One of the grand scientific challenges for realizing a sustainable personal replicator network (SPLiT) is to integrate information processing and material production from the nano- to the macro level.

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 shares. This vision is not yet science and 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)[23] 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 computer controlled 3D printer, which is able to control micro-fabrication, in part through molecular self-assembly and/or potentially atomic level controls, in order to build macroscopic structures of arbitrary complexity and composition. 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.[24] 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. One of the grand scientific challenges is discussed in Figure 5.

Some of the earlier and 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.[25] Common to these projects is an investigation of how to create and utilize living processes in a variety of hybrid biochemical, computational, and robotic systems. As our technology becomes more life-like, 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.[26]

5. ACKNOWLEDGEMENTS

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

[26] ISSP: Initiative for Science, Society and Policy, see http://science-society-policy.org under living technology.