Comparing and contrasting functional blueprints for simple self-replicating protocellular and robotics systems

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Abstract

A fundamental assumption of Artificial Life is that living processes can be supported by different material. An obvious question that follows is how the fundamental blueprint for living processes is determined by its material basis. This is the question we explore here as we compare and contrast the functional blueprints of a recently implemented protocellular (chemical) system (Kurihara et al., 2015) and a hypothetical self-replicating and self-assembling 3D printer (robotics) system (Buch & Rasmussen, 2014). The method we use to conduct this analysis is a graphical grammar originally developed to compare and contrast chemical protocellular systems (Rasmussen et al., 2008). So a secondary question we explore is whether this graphical grammar can be utilized for more general minimal living systems. We show that the developed grammar can generate transparent, graphical, functional blueprints although minor interpretation adjustments have to be made depending on the underlying material basis and scale. However, the needed level of abstraction required for keeping the diagrams directly understandable results in important information loss, thus calling for critical supportive information about the systems. We further conclude that the diagrams highlight obvious similarities as well as key differences between systems of different material basis and scale. Therefore we argue these diagrams are useful when exploring minimal living systems in particular when sharing knowledge across scientific disciplines.

Introduction

The development of minimal artificial life through protocells has evolved in multiple directions engaging scientists from many different disciplines including biologists, chemists, physicists, computer scientists and philosophers (Stano & Mavelli, 2015). Due to the increasing diversity of the field, a simple method was missing that would enable the community to share knowledge about progress and challenges across and within chemical, biological and computational platforms. To address this need a simple graphical grammar was created, which can be used to highlight protocellular functions within a single illustration (Rasmussen et al., 2008). Our current work explores whether this graphical language is still valid for protocellular models published in recent years as well as for hypothetical self-replicating robots. Further, we explore how the use of different materials conserve or change the functional blueprints for living and life-like processes.

Summary of graphical grammar method

The graphical components in the original language are: metabolism, information, container and environment. The metabolism is described with the letter E for Energy harvester. The energy from the environment is described as F for chemical fuel or as hν for light. The main function of the metabolism is to harvest energy from the environment and make it accessible for the protocell to drive the different reactions.

The informational system is denoted with the letter I. This can be DNA or RNA, which are the typical information molecules used in chemical protocell systems. The main function of the informational system is to directly or indirectly control and/or catalyze some of the protocellular processes.

The container is denoted with an A, because it consists of several units forming a single aggregate. The primary function of the container is to keep the other parts of the protocell together and within the system.

To mimic life the artificial living system should be able to process raw material from the environment and transform it to useable building blocks. The materials are denoted by capital letters, e.g. M for general materials, or P for proteins and L for lipids. The raw material or resources in the environment are denoted with a "p" describing that the components are a precursor for some building block, e.g. pM. If the materials are energized they are denoted with a "*", e.g. M*.

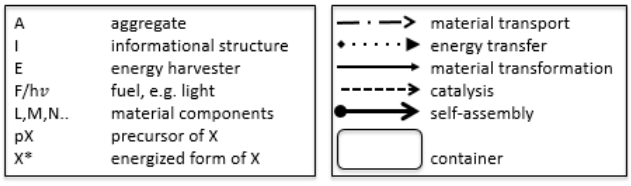

Figure 1: Elements used to depict key functionalities for minimal living and life-like system: their functional blueprint, modified version of fig 4.1 from Rasmussen et al., 2008.

Arrows are used to illustrate interactions between the different system components. Different arrows illustrate whether the interaction describes material transformation,

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energy transfer, self-assembly, catalysis or material transport, see Fig. 1.

Transformation can be a chemical reaction that causes a modification of the material such as the state from pM to M or M to M*. For such a reaction to occur the process must be driven by free energy, either carried by (one of the) reactants or added from the environment. This is shown by an energy transfer arrow.

To lower the activation energy needed for a reaction to occur a catalyst may be added to the system. As catalysis does not directly apply energy to the system it is marked by a different arrow than energy transfer.

Self-assembly are spontaneous processes that organize aggregates and are shown by yet another arrow. The last symbol in the functional blueprint is a closed line used to indicate a spatial container – an enclosure or concentration within a give area, which may be within a vesicle, an oil droplet, or on the surface of some aggregate, see Fig. 1.

Functional blueprint for recursive vesicle-based protocell with cell cycle

This life-cycle of the chemical protocell by (Kurihara et. al, 2015) is characterized by: (a) Externally pH mitigated fusion of the protocell with a giant vesicle (of opposite external charge) containing new resources to be utilized by the protocell membrane as well as the internal processes. (b) The protocell itself is also a giant vesicle that on the inside has a replicating DNA complex mitigated by an external temperature cycle. (c) The DNA together with an amphiphilic catalyst from a synthetic membrane lipid function as a metabolism and produce yet another lipid that triggers the protocellular container replication.

A graphical representation of resource "feeding" of the protocellular life-cycle is depicted in Fig. 2.

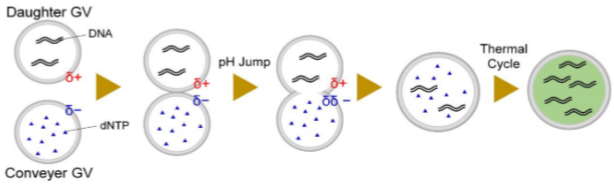

Figure 2: Fusion of resource cell (conveyer giant vesicle) with protocell (daughter giant vesicle) due to attraction caused by opposite surface charges (Kurihara et al., 2015, fig. 1.a).

The life-cycle is initiated by the container replication, which is started by adding a synthetic lipid precursor pL* to the protocellular membrane, which is composed of two phospholipids PG and PC as well as L[1]. PG is anionic, PC is a zwitterion and L is cationic. pL* can under appropriate conditions split into a cationic membrane molecule L and a waste electrolyte molecule w. Due to the composition of charges in the membrane the DNA complex embeds itself into the membrane, where it catalyzes the splitting of pL* to create L, which integrates into the membrane. The conformation of the container membrane changes gradually as L is formed, and together with the DNA complex this causes a budding formation, which eventually creates a new protocell. A continued container division will eventually create protocells depleted of materials, which must therefore be added to the cell by fusion with the resource container.

The initial attraction between the two vesicles (resource vesicle and protocell vesicle) is caused by the different surface charges on the two membranes due to different composition of lipids. As the resource container is rich on PG, low on PC, and does not contain L, the membrane will have an overall negative charge. Depending on the amount of L in the protocell the overall charge could be positive. However, to ensure a positive charge of the protocell the pH is lowered to 3, as PC becomes positively charged under acidic conditions. The fusion process is incubated for one day after which pH is restored to 8. The pH is responsible for the change in proton distribution, which is necessary for fusion, and is therefore denoted as transferred energy rather than material.

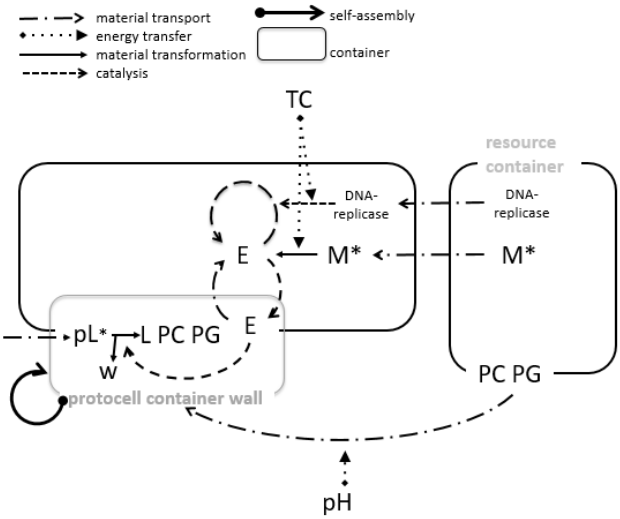

Figure 3: Functional blueprint diagram of vesicle-based protocell with a primitive life-cycle (Kurihara et al., 2015). The diagram depicts the key features of the involved resource feeding, metabolism and self-replication. The DNA complex, E, is replicated inside the vesicle, catalyzed by a replicase and fed by nucleotides from the conveyer vesicle. The marking of the protocell vesicle and conveyer vesicle (the containers) show the boundary between the cells and their environment. pL=synthetic precursor lipid. PC, PG and L[1], w = waste molecule, E = metabolic DNA complex, TC = thermal heat cycle, M = dNTP, pH = pH cycle.

DNA precursor from the conveyer is added as deoxy-ribo-nucleoside-tri-phosphate, M. M is incorporated as part of E via thermal heat cycles (TC). To do this PCR reagent was added to the protocells, which were placed in a thermal heat cycler temperature varying between 68°C and 94°C

[1] PC = 1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine, PG = 1-Palmitoyl-2-oleoyl-sn-glycero-3-phospho-rac-(1-glycerol) and L = two-tailed tetraalkylammonium-type amphiphile with dodecanyl and p-formylphenyloxy-dodecamethylene chains, w= [2-(40-aminophenoxy)ethyl]trimethyl ammonium, K. Kurihara, et al. (2010). The membrane also contains cholesterol for stability reasons, which is not discussed further here.

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respectively. DNA-replicase was added due to fusion by the resource container. Also DNA-ase was added to the environment to decrease PCR products in the environment (Shohda et al., 2011) which is not discussed further here. As M* is added to E, E is produced (replicated through template directed replication catalyzed by replicase), and can catalyze the reaction of pL* to L as described earlier.

All described information about the system is used to create a single FB illustrated in Fig. 3. From the figure, it is possible to depict how the resource container provides materials which can be integrated in the protocell, and how the protocell can transform and integrate raw material(pL) into the cell system. In figure 2 it is illustrated how the different surface charges of the protocell and resource container causes the cell to fuse. Although this is not directly shown on the FB, it is indicated by a pH energy transfer. This is used to illustrate how the lowered pH provides proton distribution necessary for fusion, but it fails to show directly how the surface charges are the primary reason for the fusion. The same is applicable for the thermal heat cycle causing the DNA to replicate, which is also marked by an energy transfer arrow to indicate that the thermal heat cycle is necessary so monomers can be added to the system. In general, the FB does not provide information about cell dynamics, which is one of the flaws of having all the information in one diagram.

However, the FB diagram clarifies how the key components interact as well as their functions. Note in Fig. 3 that the DNA complex (E) functions as a *catalyst* for the metabolic turnover of one of the container lipids, which in turn drives the container replication. Thus, the DNA complex does *not* function as a carrier of information to be translated into proteins as we see in modern biology. Using a DNA base as a catalyst is also used in other protocellular systems (DeClue et al., 2009, Rasmussen et al., 2016).

Both because the protocellular container is composed of several lipids and because one of the key metabolic processes occur within the container membrane, it makes sense to highlight the container wall processes in a separate box as seen in Fig. 3.

Reflecting on the diagram one could ask whether it would be possible to remove the DNA-replicase to simplify the system support? To expand the functionalities to include evolutionary optimization, or inventions, one could speculate which effect it would have to either modify the length, or the nucleobase composition, of the DNA complex? Certainly, there would be a combinatorial richness to explore. Also, it might be possible to screen for the impact of a different lipid composition, which is provided by the resource cell.

Functional blueprint for hypothetical self-replicating and self-assembling 3D printer

Recent years of rapid development of additive and distributed manufacturing, based on a variety of 3D printing technologies, has increased the interest in realizing a Von Neumann style Universal Constructor (Neumann 1966) within a 3D printer technology framework.

We may view two main approaches for embodied, macroscopic, universal constructors. In the one extreme we have systems where most of the necessary functional components are assembled bottom up by the internal constructor, from the available raw materials. This approach is exemplified by biological systems. It is difficult, however, to imagine how a macroscopic constructor, at least based on known technology, could do just that. In the other extreme, we have constructors that pick up already fully functional components and self-assemble these into a new copy of themselves. A simple version of this approach is already implemented by Hod Lipson and colleagues (Zykov et al., 2005). We choose a middle way for the constructor we implement using a functional blueprint. Some components are harvested from the environment while others are assembled internally bottom up. As the basis for embodiment we assume extrusion based 3D printer technology, see Fig. 4. Extrusion technology has earlier been used to partly self-replicate printers not capable of self-assembling. (Baechler et al., 2013).

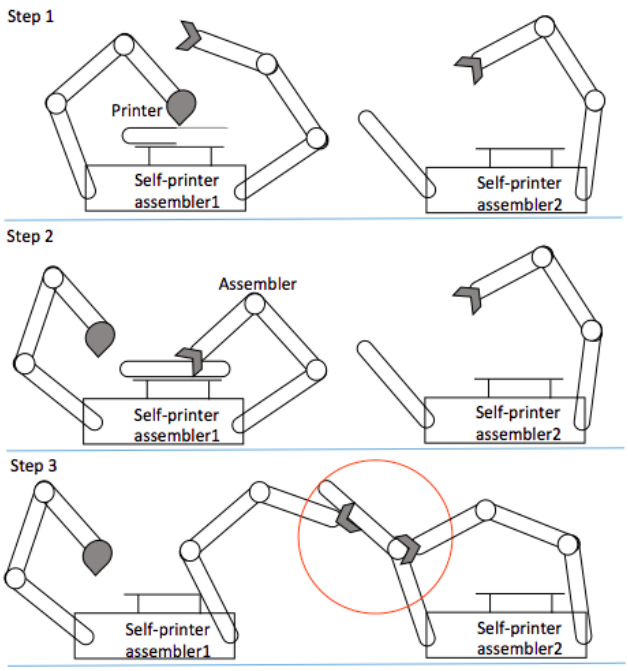

Figure 4: Schematics of a self-replicating and self-assembling printer. The printer can print material on the printing bed, which the grabbing arm(s) can transfer and assemble to make a new printer. Robotics parts, motors and some parts of the control logic (not shown, but indicated by the box) may be printed and assembled. Other parts of the control logic (including integrated circuits) should be added directly to the system and assembled by the grabbing arm. This example provides an illustration of one printer (Self-printer assembler1), which prints an arm part and hands it over to a not yet fully functional printer (Self-printer assembler2). Together the printers help assemble the arm.

The system consists of three active components and a passive scaffolding, see Fig 5. Two active parts, the printer and the assembler, may be viewed as the combined metabolism. The printer is mainly composed of extruder(s), bed and motors (E). It is powered by electricity (P) used to heat up the extruder (E*) enabling molding of pM and pN to

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create building blocks M and N, which can in turn be used to create A (scaffolding), E (printer and assembler) as well as some parts of I (computer) for new self-replicating printers.

The second active part is the assembler (also denoted E). It consists of motors and grabbing arm(s). The grabbing arm assembles the printed parts M and N plus the parts X, Y added from the environment (e.g. integrated circuits).

The metabolic processes conducted by the printer and assembler, however, are not spontaneous. They are controlled by the computer logic (I), which is the third active part, also powered by electricity (P).

The computer control performed by I is marked by the same kind of arrows used to indicate chemical catalysis, as computer process control and molecular catalytic control may be considered similar in nature. Both catalysis and computer control ultimately regulate the flow of free energy that drives the involved systems and may thus both be viewed as kinetic controls (Birch et al., 2015, Montévil & Mossio, 2015, Winning and Bechtel, 2016). The same argument applies when the assembler moves the building blocks M, N, …, X, Y, … to build a new 3D printer.

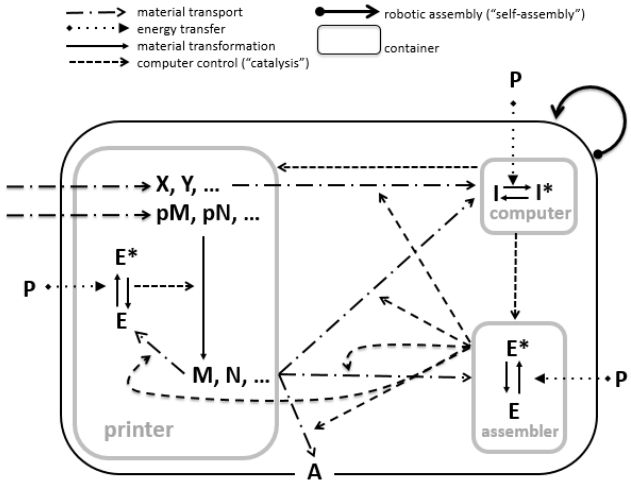

Figure 5: Functional blueprint for self-printing and self-assembling electromechanical system (Buch & Rasmussen, 2014). The figure illustrates a diagram for a hypothetical system with the ability to produce most of its own building blocks and assemble itself. P = power applied by electricity; X, Y, … = materials from the environment integrated in the system without modifications (e.g. integrated circuits); pM, pN, …= raw material added from the environment; M, N, …= printed material molded to be used as building blocks. A = scaffolding; Self-printer E = printer head, bed and motors, Self-assembler E = motor and grabbing arms, Computer: I = Informational control logic.

The same type of arrows previously used for self-assembly for molecular systems now also indicates the building / assembling of the new printer. Obviously, the involved electro-mechanical processes are active (requires free energy, is not spontaneous as molecular self-assembly) and will instead be referred to as "robotic assembly".

The resulting overall self-replication process is indicated by the circular arrow in the upper right corner of the system, see Fig. 5. Note that all materials are added in one place and then distributed to the assembler, printer, scaffolding complex and computer. The placement of the different materials made it difficult to visualize that all the printed parts go through the same 3 processes; printing, assembling and computer control and active/inactive state provided by the power source. In attempt to illustrate this more simply an alternative blueprint was created, see Fig. 6. Although the alternative FB may provide a more general overview of the different states the materials go through, all the overlapping arrows confuses the eye making the FB most applicable when different colors are added to the system.

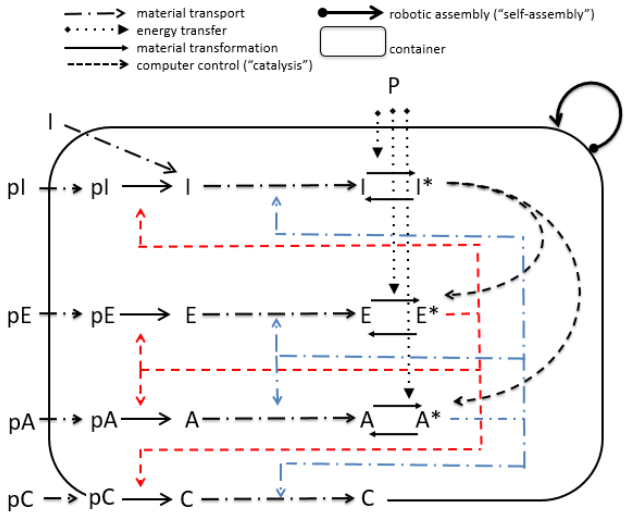

Figure 6: Alternative functional blueprint (see Fig. 5) for self-printing and self-assembling electromechanical system (Buch & Rasmussen, 2014). The figure illustrates a diagram for a hypothetical system with the ability to produce most of its own building blocks and assemble itself. P = power applied by electricity; I = materials from the environment used in the system without modifications (e.g. integrated circuits); pE, pA, pC = raw material added from the environment; A, E, C, I = printed materials molded to be used as building blocks; E = printer head, bed and motors (self-printer); A = motor and grabbing arms (self-assembler); C = scaffolding; I = informational control logic (computer).

Comparing and contrasting chemical & electromechanical systems

Comparing and contrasting minimal living and life-like systems of chemical and electromechanical nature is challenging first and foremost because these systems are studied within different scientific and engineering disciplines. The inherent need for interdisciplinary knowledge to study life and life-like processes across scientific boundaries is perhaps the greatest challenge for the Artificial Life community. The developed functional diagraming language may in a small way help this much-needed interdisciplinary communication.

Our first observation is that that it makes sense to use the four fundamental components: metabolism, information, container and environment as well as the different means of

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component interactions to describe the fundamental workings of these two very different systems. However, our analysis showed that the notion of "chemical catalysis" had to be modified to "electronic control" for the robotic system. These two different forms of interaction, however, are similar in nature as they are ultimately kinetic controls of the free energy flows that drive both systems (M. Montévil & M. Mossio, 2015, Winning and Bechtel, 2016, Bich et al., 2016). We note that in all machines - incl. living machines - we find constraints that channel the free energy that drives the machine in appropriate ways. On top of these constraints we have internal kinetic controls (e.g. catalysis and computer control) that further regulate and fine-tune the flow of free energy.

Further, "chemical self-assembly" had to be modified to "active (robotic) assembly", which is a more fundamental difference because the first is spontaneous process (thermodynamic down hill) while the second is not spontaneous and requires available free energy. In a similar way diffusive transport plays a major role for molecular scale systems (spontaneous), but has to be replaced by active transport for macroscopic systems (requires free energy). However, active transport is also prevalent for modern subcellular systems, so in that case the transport processes are thermodynamically similar at the two scales.

As we compare the diagrams in Figs 3, 5 and 6, it is clear that they share fundamental, functional, design features. Thus, the level of abstraction seems to be appropriate for the purpose of comparing systems across different material substrates and scales. However, it is also clear that each diagram cannot stand alone, because their required abstraction level means loss of significant, detailed, system information. Therefore Figs 2 and 4 are used to provide a more detailed understanding of the particular system.

It is challenging to construct FBs for a self-printing and self-assembling macroscopic system because such a system is hypothetical. This means that the components and building blocks cannot yet be specified in details. If they were we would already have resolved the issue about how to construct such a system. The functional blueprint illustrates how some of the building blocks (pM, pN, …) are printed and others are added directly from the environment (X,Y, …), but it fails to show how they are modified by the printer and whether more than one extruder is needed (to mold different materials), whether some motors should be printed while others should be supplied from the environment, etc. Also the computer controls (catalysis) would be distributed by wires, which are not shown.

These incompliances could presumably be resolved when more concrete progress is made towards creating self-replicating electromechanical systems. And perhaps these systemic diagrams can actually help us fill in the design gaps and implement novel self-replicating and self-assembling electromechanical systems. Similar systemic diagrams helped us design the Los Alamos Bug (Rasmussen et al., 2003).

Conclusions

By comparing and contrasting the functional blueprints of a recently implemented protocellular (chemical) system (Kurihara et al., 2015) and a hypothetical self-replicating and self-assembling 3D printer (robotic) system (Buch & Rasmussen, 2014), we have demonstrated that: (i) The fundamental functional blueprint that describes how the interacting components are organized for minimal living processes seems to be similar across different material platforms. (ii) The interpretation of the involved interactions has to be adjusted to the material basis and scale, while some interactions are of a similar nature while others are of a fundamental different nature. Obviously and most importantly, self-assembly and material diffusion cannot have the same critical importance for macroscopic systems. In praxis a slight change of the definition of the arrows had to be made so they could be applied to the model a self-printing and self-assembling electromechanical system. (iii) Some of the aspects of the involved systems are difficult to depict in a single abstract diagram, in particular dynamical properties. Thus additional explanatory time-resolved diagrams have to be developed.

In conclusion, both systems fit within the graphical approach without major modifications, and both FBs correctly describe the overall functions and processes of the two systems. We therefore conjecture that the presented approach is not only useful for comparing and contrasting different chemical protocellular systems, but also useful for comparing and contrasting living and life-like systems implemented in different materials and across platforms within the Artificial Life as well as the wider scientific community.

Acknowledgements

We are grateful to an anonymous reviewer for noting how we may view catalysis and computer controls as similar kinds of kinetic controls of the involved energy flows. These issues were further clarified in personal communications with Alvaro Moreno and Bill Bechtel. We also acknowledge the students, in SDU course NAT809 on Minimal living systems, for critical discussions at the onset of the presented work.

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