Synthetic Biology and Artificial Intelligence: Grounding a Cross-Disciplinary Approach to the Synthetic Exploration of (Embodied) Cognition*

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Recent scientific developments—the emergence in the 1990s of a "body-centered" artificial intelligence (AI) and the birth in the 2000s of synthetic biology (SB)—allow and require the constitution of a new cross-disciplinary synergy, that elsewhere we called "SB-AI." In this paper, we define the motivation, possibilities, limits and methodologies of this line of research. Based on the insufficiencies of embodied AI, we draw on frontier developments in synthetic cells SB to introduce a promising research program in SB-AI, which we define as *Chemical Autopoietic AI*. As we emphasize, the promise of this approach is twofold: building organizationally relevant wetware models of minimal biological-like systems, and contributing to the exploration of (embodied) cognition and to the full realization of the "embodiment turn" in contemporary AI.

Keywords: autopoiesis; embodied AI; lipid vesicles; minimal cognition; SB-AI; synthetic biology; synthetic cells

*Reviewed and accepted for publication by Hector Zenil.

1. Synthetic Biology and Artificial Intelligence: Toward a Synergy

The idea of a synergy between artificial intelligence (AI) and synthetic biology (SB) is not new and, in a sense, has always been connected with the notion of robot. In the 1920 play *R.U.R. (Rossum's Universal Robots)* [1], usually recognized as the science-fiction masterpiece from which science and engineering inherited the term "robot," the artificial human-like beings that the writer Karel Čapek called

https://doi.org/10.25088/ComplexSystems.27.3.199

"robots" were chemical machines. Unlike the robotic platforms on which the scientific exploration of intelligence has been mainly focusing its efforts since the times of (proto-) cybernetics [2], Rossum's robots were not mechanical, but biochemical. Rossum, their maker, produced them through chemical synthesis, finding a new "way of organizing living matter" that led him to generate (a singularly complex form of) natural-like intelligence [1].

A century after Čapek's fictional integration of AI and SB, the idea of applying biochemistry to the artificial modeling and recreation of natural intelligence is starting to move from science fiction to cross-disciplinary frontier research. The activation of this transition, currently at its early phases, can be traced back to two main relatively recent events in the contemporary evolution of science. The first is the birth in the 1990s of a "body-friendly" [3]—that is, a "biology-friendly"—cognitive science (CS). The second is the development, between biology and engineering, of a sci-tech research domain dedicated to the construction of biological-like systems.

Embodied AI—The "new" CS, often labeled as "embodied" [4], has been generated by the 1980s crisis of the so-called "classical" or "computationalist" CS. Its distinctive novelty is a positive focalization on the role(s) played by the biological body in cognitive processes, often described in terms of an attempt to overcome the mind/body dichotomy that defines both traditional philosophy of knowledge and classical CS. The shift from the old "naked" to the new "embodied mind" [5] has engaged contemporary CS in a multiple process of self-transformation, which has involved changes in its choices of objects of investigation, models of reference and research questions, as well as directions, approaches and methods of inquiry. Although the depth of this broad metamorphosis is nowadays legitimately questioned [6], it is undoubted that the emergence of embodied CS has profoundly impacted research in AI.

The proponents of "embodied AI"—starting from its pioneers, such as Rodney Brooks, Rolf Pfeifer and Luc Steels—reject the classical or computationalist AI approach that identifies artificial models of natural cognitive processes as purely "software models"—programs for computers reproducing cognitive performances observed in living (and primarily human) beings. The driving idea of the embodied approach to AI is that, in order to successfully explore natural cognitive processes through the construction of artifacts, specialists in CS have to build and experimentally study the adaptive behaviors of "complete" or "embodied agents," that is, functioning interactive machines incorporating biologically informed theses on adaptation and cognition. In other words, not programs, nor virtual agents, but biological-like robots: biologically inspired artificial systems endowed with bodies that dynamically embed them in environments of interac-

tion. This embodiment turn in AI, dating back to the late 1980s, until now focused on the implementation of embodied, or complete, agents as mechanical robots.

Such a programmatic transition from "software" to "hardware models" of natural cognitive processes surely represents for AI a significant embodiment-oriented novelty, but a novelty with the hint of a revival. The switch of focus it brings into the artificial modeling of cognition does not simply enable AI to cross the limits of the computationalist approach, extending its research to bodily and environmental aspects of cognition based on insights from biology. The shift from programs for computers to biological-like mechanical robots also reorients AI back toward its cybernetic origins and, more precisely, toward the original project of cybernetics. That is, structuring a unified study of biological systems and machines and attempting to overcome the divide between the inorganic and the organic world.

Synthetic Biology—New possibilities of development have been recently prepared for this project by the second of the scientific advancements mentioned. We refer to the frontier advances reached during the last decades by SB [7, 8], which now allow it to pursue not only applicative but also genuinely scientific purposes. In other words: not only bio-engineering products, but also a deeper scientific understanding of life. Presently emergent techniques of chemical synthesis and assembly, applied to biological processes, parts and systems, make SB capable not merely of modifying extant biological cells but also, and more interestingly, of building "synthetic cells." These can be realized via synthetic genome transplantation [9, 10] or from scratch, through the construction of "chemical models" of primitive cells [11, 12]. The latter approach, aiming at putting living systems together starting from biochemical molecules, is particularly relevant for the potential integration of SB and AI, since, in a sense, it provides opportune methodological and experimental preconditions to their cross-fertilization.

Indeed, on the methodological side, this approach to SB can be recognized as an innovative implementation of the research method characterizing AI. This is a methodological strategy that AI inherited from cybernetics and since the 1950s has been applying to the study of cognition through the realization of software and hardware models of natural cognitive processes. Today it is often called the "understanding-by-building" method [13] and thematized as the methodological approach supporting inquiries that aim at contributing to the scientific understanding of natural processes through their "artificial" or "synthetic" modeling. In a few words: the construction and experimental manipulation of artifacts that, reproducing target natural processes on the basis of scientific hypotheses, can be considered "material models" of these processes, useful for experimentally

https://doi.org/10.25088/ComplexSystems.27.3.199

testing the hypotheses they express. Practitioners in SB explicitly indicate it as their methodological approach when studying life based on the construction of "chemical models" of biological processes. These models, often defined as "wetware models" [14], can be considered a third kind of model of living processes that, together with software and hardware models, current science produces through the understanding-by-building approach.

SB-AI—This methodological affinity, together with the need of embodied AI to establish synergies with the life sciences, opens directions of cross-disciplinary cooperation between SB and AI that appear far from meeting the ambitions of Čapekian-like science fiction, but promise to produce concrete insights.

On the experimental side, in SB the understanding-by-building approach generates for science the unique possibility of empirically studying processes of emergence of minimal living organisms through the synthesis of artificial chemical systems. As we will argue, when SB succeeds in incorporating in these systems self-regulating mechanisms of biological self-organization and self-production, these artifacts can be of high scientific interest for AI. Once they are situated in appropriate environments of interaction and/or enabled to interact with other synthetic or natural biological systems, these artifacts open the possibility of designing experimental scenarios useful for studying adaptive dynamics in minimal biological-like embodied agents that are not mechanical, but chemical. In other words, they open the possibility of SB-AI cooperative explorations of minimal forms of natural-like embodied cognition: experimental inquiries based not on hardware, but on wetware models. This undertaking would multiply the ways in which the sciences of the artificial can contribute to the study of embodied cognition. They would allow these sciences not only to hybridize hardware and wetware models, but also to cross-fertilize the levels of inquiry generated by these two different models of embodied cognitive processes and agents.

In this paper, we would like to define the basic lines of a cross-disciplinary SB-AI approach for the scientific study of minimal (embodied) cognition grounded in autopoietic cognitive biology (cf. Appendix A), that is, a theoretical perspective that would contribute to not only radical trends of embodied CS [5, 15], but also frontier approaches in the synthetic cells research line in SB [16–19]. In Section 2, we focus on frontier SB approaches, illustrating interesting avant-garde developments that make them relevant for embodied AI research. In Section 3, we illustrate in what sense embodied AI could profit from the development of these SB approaches in its research domain and, in particular, we draw the basic lines of a nascent SB-AI research direction that in previous work we introduced as "chemical autopoietic AI" [20].

2. A Synthetic Biology Approach to Embodied Artificial Intelligence

2.1 A Short Historical Note

SB is probably the most relevant novelty in the biological experimental sciences since the 2000s. Born in the United States as a way to "engineer biology" [8, 21, 22], SB has rapidly attracted the interest of a large number of scientists due to its power and versatility. The SB starting point is to consider biological organisms as systems composed by interconnected parts, in analogy with electronic systems composed by individual components, as represented in Figure 1 [23].

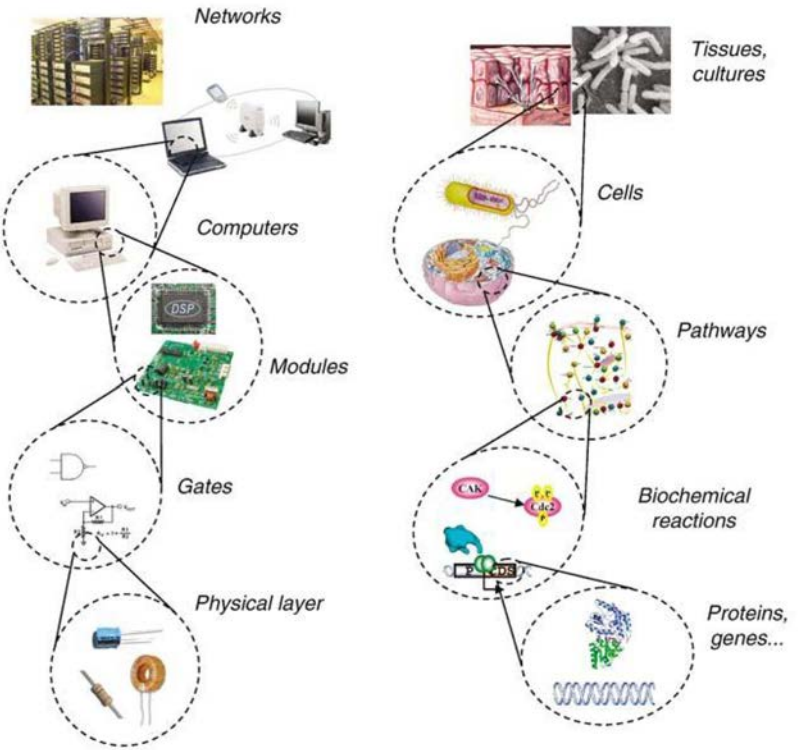

Figure 1. A possible hierarchy for SB is inspired by computer engineering. Reproduced from [23] with permission of Wiley.

This parallelism is made stronger thanks to the concept of "standard" biological parts (or bio-bricks, see: parts.igem.org), referring to DNA standard sequences that can be connected in a standard way with each other, allowing biological engineers to build genetic circuits as electrical engineers do electronic circuits. The background cellular metabolism and the core transcription-translation machinery become the "chassis" where engineered biological parts are grafted,

implemented, added, eliminated, duplicated and modified. This is generally done *in vivo*, by exploiting the power and the robustness of (micro)organisms. In this way, cell processes are quantitatively examined, redesigned and rewired to achieve particular purposes. The latter are generally set up *a priori* for achieving a specific goal, for example, redirecting the metabolism toward the production of useful compounds such as fine chemicals, biofuels, drugs, reporter proteins and so on.

In addition to this "mainstream" approach, there is a second SB wave that considers instead the possibility of constructing very simplified (minimal) cells from scratch [12]. This approach, which is now recognized as the "bottom-up branch" of SB [24], actually preceded the SB advent by many years, because its roots are deeply anchored to bionics, biomimetics and the exploration of cellular models. In other words: to approaches relying on the previously cited understanding-by-building.

Deamer and Morowitz [25, 26] and later Luisi [27] contributed to the promotion of the term "minimal cell." In particular, in the 1990s Luisi and collaborators decisively started a long-term and wide investigation on minimal cells construction in the laboratory, laying the foundations of contemporary approaches [28–32]. Even more importantly for the present discussion, the Swiss group adopted the theory of autopoiesis (cf. Appendix A) as its reference theoretical framework for the construction of synthetic minimal cells. Based on this choice, an important fusion between theory and experiments was realized, as lucidly described in a programmatic article by Luisi and Varela [33]. This powerful combination is highly appreciated in the agenda of contemporary synthetic cell studies. Our efforts and contributions, here and elsewhere, aim at fully developing this connection, which can be very fruitful for both basic and applied science (see Section 3).

2.2 What Are Semi-synthetic Minimal Cells?

Within this framework, the key question is how to build synthetic (or semi-synthetic) cell-like compartments that exhibit the essential traits of biological cells, in particular, their autopoiesis (cf. Appendix A).

Synthetic cells should display specific properties and perform those functions that ultimately have to bring about the construction of all their components from within. This behavior should be achieved despite the strong reduction of complexity that necessarily follows from their laboratory origin. The creation of living synthetic cells is the actual long-term goal of this enterprise and still appears out of reach. On the other hand, nonliving synthetic cells would work well (or would be even preferable) for most biotechnological applications. It should be noted that such manmade "minimal" cells somehow

resemble primitive (ancient) cells before the complexification generated by evolution: they function as primitive cell models as well. The whole research on minimal synthetic cells is, therefore, related to the two previously mentioned, apparently distant goals. In the first one, minimal cells are intended as exploitable systems for biotechnological applications; in the second, they are intended as primitive cells; the common traits refer to the operational procedure for their construction (Figure 2).

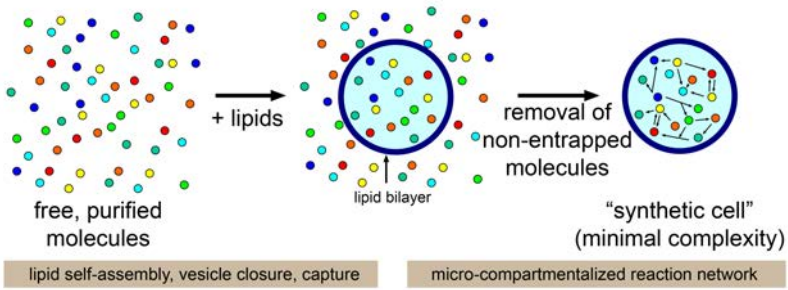

Figure 2. Building synthetic cells. The operational procedure to construct synthetic cells consists in the encapsulation of individual molecules inside a lipid microcompartment, generally a lipid vesicle (liposome). To this end, lipids are allowed to self-assemble in the form of a closed bilayer membrane, so as to constitute a simple cell-like compartment, whose size typically ranges from 0.1 to tens of μm. Solutes are randomly captured by the vesicle in the moment of its closure and do not escape, because the lipid membrane is a tightly sealed boundary (only small uncharged molecules can permeate it). Next, non-entrapped, free molecules are removed or their function is abrogated, in order to have a cell-like system where internalized molecules can react, providing a useful cellular mimic. Through the proper choice of the molecules to be encapsulated, it is possible to design synthetic cells for any specific purpose. Note that the scheme represents the most basic method for solute encapsulation. To date, however, other strategies are applied—in particular, the droplet transfer method [34] and microfluidics-based processes [35].

But there is a third research line, strictly connected to the theory of autopoiesis (cf. Appendix A), that should be developed, as we argue. This interrelates SB to embodied AI research, as it focuses on the synthetic modeling of (minimal) embodied cognitive processes through the construction of synthetic minimal cells. This novel avenue, in a sense, is a natural prolongation of synthetic cell research, which, since its origins, has been deeply coupled with autopoietic biology.

As mentioned, semi-synthetic minimal cells (more briefly: "synthetic cells") are built by incorporating molecular elements such

as DNA, RNA, proteins, ribosomes and others inside a synthetic microcompartment); see Figure 2.

Synthetic cells are composed by well-characterized parts, which can be collected from several different organisms. These sorts of cells are assembled according to modularity, orthogonality and programmability principles. The encoding DNA is built following the bio-brick philosophy, leading to a programmable behavior. Thanks to well-known methods for genetic and metabolic control, genetic circuits can be designed in order to generate precise outputs in the chemical domain (e.g., high or low concentration of a certain chemical). Such a design is often computer supported and then optimized in the laboratory.

Synthetic cells are based on the convergence of cell-free technology and liposome technology. Biochemical pathways can be reconstructed inside liposomes. Protein synthesis is one of these highly relevant pathways. From a practical perspective, the success of synthetic cell construction is based on the capability of preparing liposomes filled with (or whose membrane is decorated with) molecular components of interest. In the past, liposome technology was mainly developed for drug delivery applications [36], whereas new protocols are specifically developed for synthetic cell technology (think, for example, of microfluidics). Currently, the combination of cell-free systems with microfluidic devices is under intense development [37] and soon will allow the preparation of solute-filled liposomes in a highly controlled and reproducible manner.

The principal achievements of synthetic cells research have been widely discussed by us and by others [38–42], together with its possible future directions. Here we would like to recapitulate only three major aspects that are connected with our vision and the related research project.

First—Historically, the theoretical canvas for a synthetic cell design has been the theory of autopoiesis by Maturana and Varela [17, 43]. This theory deals primarily with the question, What is life?, which it answers by defining the specific form of dynamical organization that characterizes all biological systems (see Section 3 and Appendix A). A historical account of the origin of the cross-fertilization between chemistry and autopoiesis, based on the scientific partnership between Luisi and Varela, can be found in [44]. It is interesting to read that:

> I [Pier Luigi Luisi] was leading an experimental research group at the ETHZ, working with self-organization and biopolymers, and with Francisco, we began to look for experimental systems capable of showing autopoiesis. We spent much time thinking of water structure and its flickering properties, but nothing came out of this. However, something came from my studies on

> reverse micelles, the small spherical structures formed by surfactants in apolar solvents and having an internal water pool where hydrophilic reactants can be incorporated, and we were able to conceive an autopoietic system based on the idea… [44].

Indeed, such an idea was published in 1989, in a programmatic paper [18] followed by several experimental reports (reviewed in [45]). Note that Maturana and Varela had already attempted, unfortunately with limited success, the construction of autopoietic systems in the "molecular protobion" project [46].

Second—Initial work in SB was successfully devoted to the self-production of autopoietic units, by adopting reverse micelles, micelles and vesicles. A typical example is given by autopoietic self-reproduction of reverse micelles [47]. These are tiny water compartments suspended in apolar solvents like hexane and surrounded by a surfactant layer that can host a water-soluble reactant. If a proper precursor is added to the hexane solution, so that the precursor is chemically transformed into the reverse micelle surfactant, a sort of autopoietic mechanism is observed. The reverse micelles synthesize their "boundary" from within; they grow because of this surface enlargement and eventually divide. Similar mechanisms can be achieved with micelles [48] and vesicles [49]. It is important to note that these artificial chemical systems were extremely simple, which is a plus when the target is the modeling of very primitive cells. However, when the focus shifts from primitive cells to minimal cells of nontrivial complexity—yet enormously simpler than biological cells—the construction of autopoietic self-reproducing cells becomes significantly challenging. This steep barrier is due to the sophistication of modern molecular components, which display high functional performances at the expense of simplicity.

Third—Proteins can be synthesized, in functional form, inside liposomes by cell-free transcription-translation (TX-TL) kits [39, 50, 51] (Figure 3). TX-TL reactions are central to the cellular metabolism. It has been calculated that about 50% of the minimal genome deals with genes involved in TX-TL processes [52]. Therefore, performing such reactions inside liposomes is a key step in the development of synthetic cells. There are two types of TX-TL kits: cell extracts (from bacteria or eukaria) and reconstituted systems. The latter are particularly useful for bottom-up SB approaches because their composition is perfectly known, adjustable and—especially—minimal. The currently available minimal and reconstituted TX-TL kit (based on bacteria ribosomes and proteins) is the PURE system, available since 2001 [53]. It was first incorporated inside liposomes in 2006–2008 [54–56]. Why is protein synthesis important? Via TX-TL reactions, synthetic cells can produce enzymes, receptors, membrane channels,

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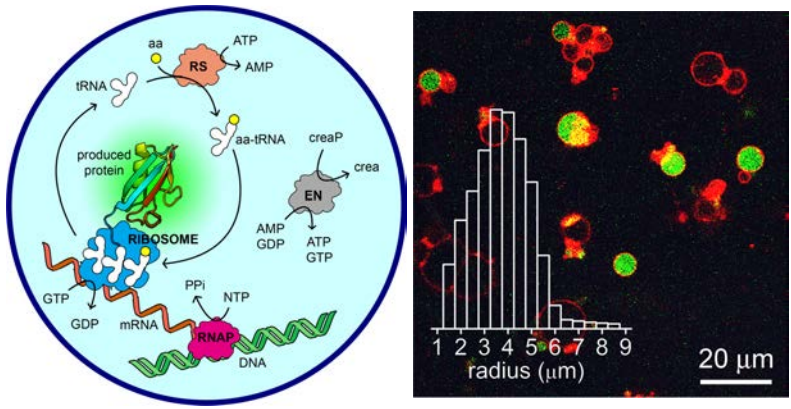

(a) (b)

Figure 3. TX-TL reactions inside lipid vesicles (liposomes). (a) Schematic representation of protein synthesis. TX-TL kits perform protein synthesis and their operation can be broken down into four "modules," namely transcription, translation, amino acid charging and energy regeneration. The reactions of each module are schematically rendered in the graphic. Transcription (TX): production of mRNA from DNA template and nucleoside triphosphates (NTPs); the catalyst is RNA polymerase (RNAP, in magenta). Translation (TL): production of protein from mRNA template and aminoacyl-tRNAs (aa-tRNAs), using GTP energy; catalyst: ribosome (blue). Amino acid charging: synthesis of aa-tRNAs from amino acids (aa) and tRNAs, using ATP energy; catalyst: aminoacyl-t-RNAs synthetases (RS, in orange). Energy regeneration: recharging GDP and AMP to GTP and ATP at the expense of phosphocreatine (creaP), and similar reactions; catalysts: a set of kinases (EN, in gray). (b) Green fluorescent protein (GFP) synthesis inside giant liposomes (mean radius: 4 μm) as revealed by confocal laser scanning microscopy (green objects). Note that not all liposomes are able to synthesize GFP; reproduced from [59] with permission from The Royal Society of Chemistry.

cytoskeletal proteins, etc. and thus perform one or more functions. The autopoietically desired function is the production of all cellular components (i.e., in this context, of all PURE system components), but to date, this goal seems out of reach. However some attempts have already been reported, focusing on ribosomes [57, 58]. As we specify later, because TX-TL reactions are under DNA control, it is possible—at least in principle—to program synthetic cells' behavior by adapting well-known biochemical regulation mechanisms, thanks to the so-called "genetic circuits." Even if this will not impact at large on the TX-TL machinery (and thus the autopoietic organization), it might represent a useful first step toward minimal self-regulation in response to external perturbations in these types of cytomimetic systems.

2.3 An Experimental Perspective?

If autopoietic minimal cells are cognitive embodied agents (cf. Appendix A), then synthetically creating simplified chemical models of autopoietic units may allow research to synthetically explore embodied cognition. This is the main assumption of our vision, which we develop in line with the experimental "nearest goal approach" typical of bottom-up SB. This approach drives bottom-up SB to construct synthetic biological-like systems that are not necessarily alive but display the lifelike features under inquiry. Assuming this approach in SB-AI research means to focus the inquiries on an analogous "nearest goal." This can be, for example, the construction of simplified minimal autopoietic cells interacting with the environment—with natural cells or synthetic cells—based on endogenous self-regulative processes.

By developing this approach, we intend to target interactions between a minimal autopoietic system and its environment, during which the system, through self-transformation, maintains its structural coupling with the environment (Figure 4). Within the system, this self-determined activity of transformation is induced by exogenous perturbations that activate endogenous dynamics of self-regulation, which implies the system's transition between different dynamical (autopoietic) states. According to the autopoietic framework described in Appendix A, this kind of activity of self-regulation, supporting the maintenance of the structural coupling with the environment, can be interpreted as a cognitive process that leads to the stable association of endogenous patterns of self-regulation with exogenous perturbing events. If the system cannot cope with a perturbation—that is, if the system cannot associate any internal pattern of self-regulation to the intervening perturbing event (shown in Figure 4 as "perturbation 1-4")—its self-transformation cannot be considered as a cognitive process. The outcome is the interruption of the system's structural coupling with the environment and the disruption of its autopoietic organization.

How can such a theoretical scenario be translated into a realizable experimental plan? The emerging techniques directed toward designing and constructing genetic networks *in vitro* [60] suggest a possible approach. In particular, if a gene expression pattern—grafted over a TX-TL homeostatic machinery fueled by constant resources—can be considered a proxy of an autopoietic state, transitions between gene expression patterns would model the transition between autopoietic states. The latter, in turn, can be thought as a sort of attractor in the sense this notion assumes with regard to Boolean genetic networks [61, 62].

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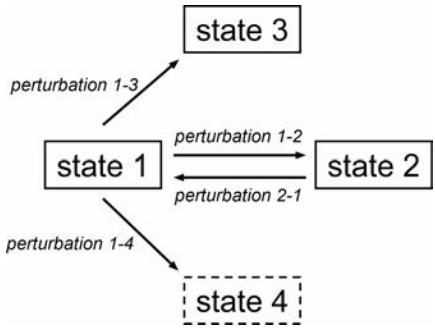

Figure 4. Schematic illustration of transitions between dynamical autopoietic states intended as generation and association of self-regulation patterns to perturbing events. The self-determined transition of the autopoietic system between different dynamical states (1, 2, 3, 4) is triggered by environmental events perturbing its self-production processes, generated by the genetic-metabolic circuitry. When the system cannot cope with a perturbation (here indicated as "perturbation 1-4"), it undergoes a transition toward a non-autopoietic state (4). See text for details.

Even if this perspective lacks a proper degree of accuracy and might appear too rudimentary when compared with the precise genetic-metabolic regulation patterns in biological cells, the match (or the similarity) between autopoietic states, Boolean genetic network attractors and minimal gene expression pattern can lead to a specific research landscape that might be useful and interesting to explore.

A major drive to develop small genetic circuits has characterized SB since its early days. Artificial parts, devices and systems are designed and examined by numerical modeling, then implemented in the form of their genetic counterpart, that is, DNA sequence(s), and inserted into living cells (typically bacteria) to drive a behavior. Let us consider two very well-known examples, which actually are foundational to SB.

- The *toggle switch* [63] consists of a genetic toggle switch based on two transcriptional factors (both repressing the expression of the regulated gene), which are assembled in order to inhibit each other (mutual inhibition, Figure 5(a)). The dynamics of such a circuit is a stable "toggle" between two states, in response to external inputs (a small lactose-analog ligand called IPTG and heat).
- The *repressilator* [64] is a slightly more complicated small genetic circuit that was engineered to display stable oscillations. Three negative-feedback loops were co-assembled in the circuit, as shown in Figure 5(b). Actually, each expressed protein acted as repressor for another in the circuit, operating on the respective promoter.

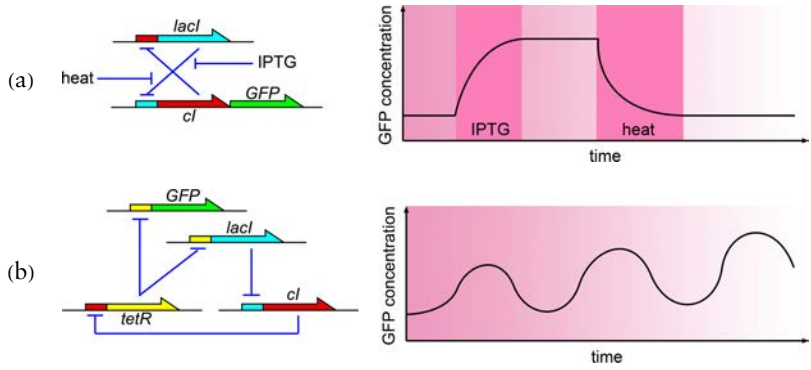

Figure 5. Two classical examples of SB circuits in vivo (2000). (a) The toggle switch [63]; (b) the repressilator [64]. These genetic circuits were designed, constructed in vitro, then inserted, in the form of DNA sequences (plasmids) inside *E. coli*. The green fluorescent protein (GFP) was used as an output protein, to determine the state of the circuit. In both cases, one can have ON and OFF states, corresponding to high and low levels of GFP production. Note that in (a), the transitions between the states are triggered by specific factors such as IPTG (a small molecule) or heat.

These two pioneer papers showed the way to design, model and implement genetic circuits from standard biological parts. Moreover, they established the typical engineering workflow in SB (namely, the iteration of "design-construct-evaluate-refine" operations).

In addition to the two mentioned examples, which have been implemented in vivo (in *E. coli*), small genetic circuits have been also implemented in vitro, relying on TX-TL kits. As mentioned by Noireaux, Bar-Ziv and Libchaber in [60], in vitro TX-TL systems present some advantages when compared to the well-known in vivo protein synthesis. The principal ones are the control of the components' concentration, the absence of unknown background processes and the easier quantitation of their pattern.

A repertoire of cell-free genetic circuits is given in Figure 6. Their function has been generally tested in the test tube, and some of them inside liposomes [65–69]. Current methods of synthetic cell preparation have essentially solved the main operative issue (how to efficiently co-encapsulate all the required components). In particular, the droplet transfer method [70] and microfluidic devices are quite suitable for such a co-encapsulation [35].

In order to function, genetic circuits need the synthesis of one or more proteins in the correct three-dimensional fold. This is achieved by several different TX-TL kits, the selection of which should be

evaluated based on a case-by-case approach. TX-TL kits are often cellular extracts, but not always. In addition to the widely used *Escherichia coli* extracts, eukaryotic systems are also well known (from rabbit reticulocytes, wheat germ or insect cells), whereas the PURE system is a fully reconstituted system that operates with the minimal number of components [53, 71].

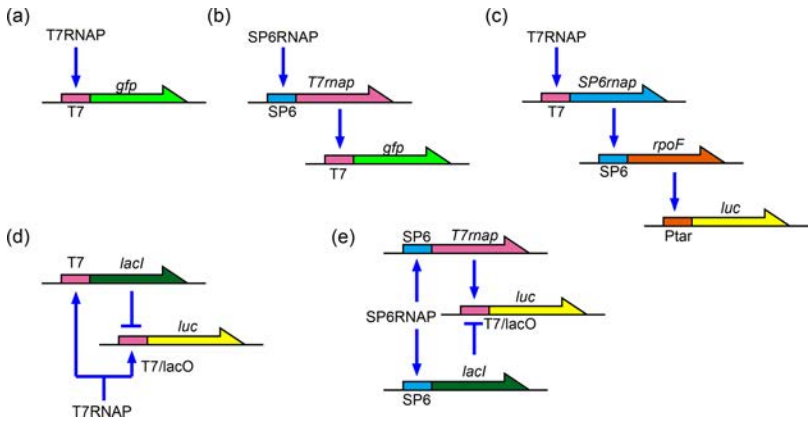

Figure 6. Examples of simple genetic circuits whose function has been demonstrated in vitro, that is, by employing cell extracts and biobricks DNA, as reported in [60]. The arrows represent DNA sequences that encode for proteins. T7, SP6, Ptar indicate the promoter region, whereas the gene names are rendered in italics (*gfp*, *T7rnap*, *SP6rnap*, *rpoF*, *luc*, *lacI*). Arrows indicate "activation," whereas T-end lines indicate "inhibition." (a–c) One-, two- and three-stage gene expression (cascades); (d, e) circuits with repression steps. More elaborated circuits based on *E. coli* RNA polymerase and sigma factors can be found in a recent work by Noireaux and collaborators [67]. To read the graphic, for example, (d), consider that T7RNAP is an RNA polymerase that starts the transcription of two genes under the T7 promoter (i.e., *lacI* and *luc*), so that two proteins (lacI and luc) are produced. In turn, the lacI protein operates as an inhibitor for the transcription of the luc gene.

These emerging possibilities of designing small genetic networks inside liposomes officially introduce SB into the domain of AI experimental research, which, historically, is rooted in the 1940s cybernetic design of Boolean networks as models of cognitive systems and processes [72–74]. The main assumption of our SB-AI approach is that today Boolean networks can play a new role in the development of AI. When built and explored by SB, they could be able to trigger significant developments supporting the full expression of embodied AI.

3. Toward Autopoietic Chemical Artificial Intelligence

3.1 A Role for a Synthetic Biology-Artificial Intelligence Approach in the Development of Embodied Artificial Intelligence

The role that the synthetic exploration of minimal forms of cognition can play in the development of embodied AI emerges when considering the dissatisfaction that, for almost three decades, has been growing among the specialists of this "*nouvelle* AI."

One of the earliest and clearest expressions of this discontent is due to Brooks, frequently recognized as the founder of embodied AI [75, 76].

Brooks's groundwork can be seen as an attempt to address the criticisms of computationalist AI advanced by the philosophical debate. Famously, between the 1980s and the 1990s, authors such as Searle [77] and Harnad [78] converged in tracing back the failure of classical AI to the "insular" character of computational cognition. Since it starts with symbolic inputs, proceeds as syntactic manipulation of symbolic information and ends by producing symbolic outputs, computational cognition does not include a phase during which the cognitive system can attribute definite semantic values to the symbols it manipulates—meanings based on its interactions with the world. Considered in this perspective, classic artificial cognitive systems appear destined to the incapability of accomplishing real-world and context-sensitive tasks, as they are "cut off" from their worlds. Their purely syntactic symbol processing, being independent from the specifics of the environmental situation, cannot be able to adaptively cope with it.

Brooks's innovative research plan proposed two explicit principles—"embodiment" and "embedment" (1991)—that responded to this deficiency of classic AI by giving to artificial cognitive systems a body and, on this basis, a world of reference. To these principles Brooks gave a "radical embodiment" inclination, which focused his research not simply on providing artificial systems with bodily structures to perceive and act on the environment, but also on designing for them a form of artificial "intelligence without representation" [75]. Brooks intended to create robots whose interactive behaviors are based on direct sensor-motor interaction, without the support of abstract descriptions or internal mappings of the environment. The idea was that of substituting classical "ungrounded" representations with sensor-motor associations—that is, sensor-motor self-regulation. Brooksian embodied and embedded artificial systems, similarly to biological systems, provided themselves information about their environment simply by being or moving in it and associating external conditions to internal self-regulative sensor-motor configurations. This

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way, in a sense, they were able to learn about their environment through their body—which computers cannot do.

Since the 1990s, this kind of embodied-oriented AI has seen significant developments, aiming at modeling in robots the whole range of natural cognitive processes, human ones included, through multiple coupling of basic sensor-motor processes. The search for biologically plausible design characterizing programmatically the Brooksian approach activated an extremely effective exploration of natural bodily and neural mechanisms based on physiological, neuro-scientific and ethological research, and has led to increasingly adaptable and autonomous biological-like (more specifically, animal-like) robots [13]. Yet, the development of embodied AI did not end the discontent that emerged with the "winter" of classical AI in the 1980s.

Already in the 1990s, the same Brooks expressed a deep dissatisfaction for embodied AI, claiming that "Perhaps we have all missed some organizing principle of biological systems […]. Perhaps there is a way of looking at biological systems which will illuminate an inherent necessity in some aspect of the interactions of their parts that is completely missing from our artificial systems" [76]. Similarly, Di Paolo [79] emphasized that what embodied AI misses are pertinent robotic models of the internal organization of living systems, which allow these systems to shape their environment into a "space of meaning"—a world whose events are charged with meanings related to the systems' "continuing mode of existence and ultimately survival." Deepening this line of criticism, Ziemke [6, 80, 81] questioned the genuineness of the "embodiment" of robots implemented by the "nouvelle AI." He pointed out that, while emphasizing that the physical body is the core of cognitive interaction between robotic agents and their niche, most of embodied AI works with software models of robotic platforms and produces robots that, being controlled by computer programs, are "still just as computational as the computer programs of traditional AI." Furthermore, Ziemke also attacked the subdivisions of embodied AI that fully eliminate computationalist representations. He showed that they share with the rest of the new AI "a mechanical/behaviorist view of the body," which cannot ground "intrinsic meanings" and "intrinsic intentionality" and is destined to account exclusively for functional aspects of cognitive behavior.

There is no space here for a detailed analysis. We have to limit ourselves to outlining the common idea of these positions, which is more or less this: the "complete agents" built by embodied AI are cognitive agents that lack a biological-like bodily organization and, thus, a body in the proper sense. Despite its focus on living organisms, embodied AI still misses a deep understanding of the role played by the biological bodily organization in generating a form of cognition that, far from performing extrinsic problem-solving, continuously

addresses the problem of maintaining the system's coherence in an ever-changing environment, by charging external perturbing events with internally generated operational meanings that support effective self-regulation.

Converging in this critical analysis of embodied AI, we believe that to fully establish the embodied approach, AI has still to realize a main shift of focus, leading from what we can define as the current "morphological" to a new "organizational" embodied AI.

Undoubtedly, concentrating on endowing robots with morphology and anatomy similar to those of living organisms is a first and fundamental step toward an effective embodied approach to AI. This ensures the possibility of making a significant part of the cognitive activity dependent on the mechanical body of robots and its interaction with the environment, as happens in biological systems. But this incipient way of overcoming the classical software/hardware dichotomy, that is, the AI mind/body dichotomy, is not enough to create a biological-like—a genuinely "embodied"—artificial mind. The biological mind does not emerge from bodily morphology and related sensor-motor self-regulation. It emerges from a self-determined and self-regulative process of self-production. To model this process into robots, AI has to endow its complete agents not only with biological-like morphology or anatomy, but also with an internal organization similar to that defining the biological body. That is: with an internal mechanism integrating diverse elements in a coordinated dynamics able to generate an organized system that interacts as a whole with its environment to conserve its own viability.

We think that SB, even when limited to exploring minimal chemical models of life, can significantly contribute to addressing this challenge, and we assign this task to autopoietic chemical AI.

3.2 The Relevance of Autopoiesis for the Evolution of Embodied Artificial Intelligence

The relevance of autopoiesis (cf. Appendix A) for the establishment of embodied AI relies on three main characteristics of its definition of life.

The first characteristic can be found in the autopoietic "synthetic" definitional approach, based on which this theory defines life by proposing not a list of properties of living systems, but a mechanism able to generate, from a multiplicity of separated components, a minimal living system and all the related biological processes. The promise of this definitional approach is that if science implements the mechanism specified, in principle it will be able to recreate the whole biological domain as we know it.

The second characteristic that makes autopoietic biology relevant for the evolution of embodied AI relies on the theoretical content of

https://doi.org/10.25088/ComplexSystems.27.3.199

its definition of life. This consists of the notion of autopoietic organization, which describes the mechanism generating minimal living systems as:

- [...] A network of processes of production (transformation and destruction) of components that produces the components that: (*i*) through their interactions and transformations continuously regenerate and realize the network of processes (relations) that produced them; and (*ii*) constitute it (the machine) as a concrete unity in the space in which they (the components) exist by specifying the topological domain of its realization as such a network [...] ([17, p. 79]).

This definition of life, focusing on biological self-production, is connected to the self-regulative activity of living systems and, in this sense, to their cognitive activity.

- It follows that an autopoietic machine continuously regenerates and specifies its own organization through its operations as a system of production of its own components, and does this in an endless turnover of components under conditions of continuous perturbations and compensations of perturbations. ([17, p. 79]).

According to this view, the activity of generation of endogenous meanings, expressed in patterns of self-regulation, for external perturbations is inseparable from the activity of biological self-production. This way, the notion of autopoietic organization incorporates the autopoietic "life = cognition" thesis, according to which "living systems are cognitive systems, and living as a process is a process of cognition" [16]. Implementing the mechanism described by this notion would allow AI to test this (controversial) thesis.

The third characteristic that makes autopoiesis relevant for the full establishment of embodied AI relies on the fact that the definition of the autopoietic organization does not specify the material components of the autopoietic network. This implies that in principle, to produce an autopoietic network, AI does not have to focus on the actual components of life as we know it, but can use all kinds of components able to generate an autopoietic network. This gives AI the possibility to implement different material models of (minimal) life and cognition, that is, (a variety of) forms of biological and cognitive systems that, with respect to their material structure, do not exist in nature.

3.3 Toward Chemical Autopoietic Artificial Intelligence: From Morphological to Organizational Embodied Artificial Intelligence

The artificial implementation of the autopoietic organization imposes significant restrictions to AI.

As we will clarify in detail in a future publication, software models (computer simulations) are abstract models of the autopoietic network, incapable of fully implementing the self-productive process described by the notion of autopoietic organization: the reciprocal

production of the material structure and the functional organization of the autopoietic system.

Furthermore, as clearly pointed out by Froese and Ziemke in 2009 [82], current mechanical robotics appear to be far from generating material models of the dynamic network of operations of reciprocal production and transformation of components described by the autopoietic definition of life.

Yet things are significantly different for SB. In fact, SB operates with components that are chemical molecules and, even when they differ from those constituting terrestrial life in their chemical structure, they do not differ from them in reactivity. Thus, in principle, SB is able to generate material models of the autopoietic network and actually is already engaged in designing primitive versions of them. Its main obstacle is the very high level of complexity characterizing even minimal autopoietic networks—especially when based on available biomacromolecules. In this sense the main challenge, for an SB-AI approach based on autopoiesis, is this: building a simplified chemical model of the autopoietic organization that can be relevant for synthetically studying the biological organization as a cognitive organization.

Our way of addressing this challenge relies on the assumption that chemical Boolean networks encapsulated into liposomes, in defined conditions, can generate "organizationally relevant" wetware models of minimal autopoietic systems, that is, minimal self-producing systems characterized by the autopoietic organization [83]. The main task to accomplish, in order to achieve this "organizational relevance," is creating liposomes that include relatively simple (constructable) Boolean networks actively participating in the process of the liposomes and their own production in a dynamic and self-regulative way, coherently with the definition of the autopoietic organization. A sketchy model of these sorts of systems is reported in Figure 7. This is a tentative design, inspired by previous work [84] and meant to give the community an essential but concrete model to test this approach. An accurate design, with a detailed study of realizability, is currently under development.

To experimentally build these kinds of self-producing "Boolean networked liposomes" would equate to having the possibility of testing and developing the autopoietic perspective on the biological cognitive coupling (cf. Appendix A.4) by trying to answer research questions such as the following: At what level of internal dynamic complexity is a minimal autopoietic unit able to establish a relation of structural coupling with the environment as it is described by the theory of autopoiesis? Can this kind of relationship be established by autonomous systems that cannot be defined as fully fledged autopoietic systems? Why? Are there significant variations in this coupling and the related activity of meaning generation with the progressive

increase of the dynamic complexity of the modeled autopoietic unit? If yes, can we distinguish and classify different kinds of cognitive coupling already at the level of minimal synthetic autopoietic units? Can we do this with regard to the coupling that can be established between different artificial—or between artificial and natural—minimal autopoietic units?

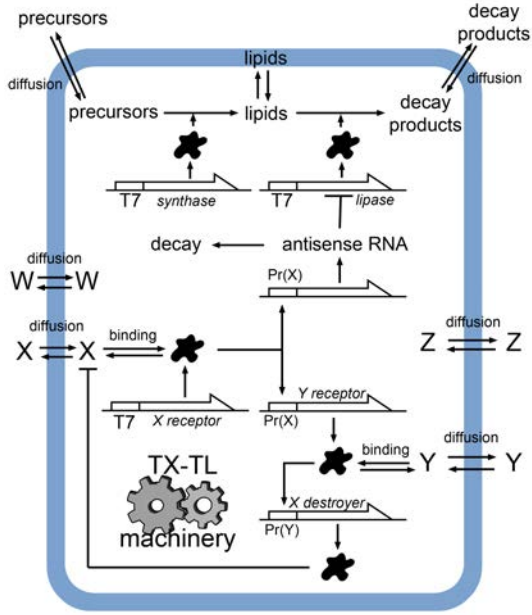

Figure 7. Hypothetical design of an SC that changes its behavior by modifying gene expression pattern. The aqueous core of a lipid's vesicles is filled with the TX-TL machinery (ca. 100 different molecules) and six genetic elements. Two genes encode for proteins that operate on lipids (synthase and lipase), whereas a third gene encodes for a receptor for the molecule X. These three proteins are synthesized "constitutively" in the liposome, which, as a result, is in a homeostatic state (the membrane lipids are synthesized and degraded at similar rates). If X molecules appear in the environment and freely permeate the lipid membrane, they can be "perceived" by the network and activate the synthesis of two more genes. One produces an antisense RNA molecule that inhibits the synthesis of the lipase, the other produces a receptor for the molecule Y. As a result, lipid degradation is stopped, and the liposome grows because its membrane grows. If Y molecules appear in the environment, they can be perceived by the network, so that the production of an enzyme that degrades X molecules is produced. This will cause an interruption of the synthesis of the antisense RNA. Its concentration will decrease by a spontaneous decay reaction, and the lipase will be synthesized again, restoring the homeostatic state. The network is able to select X and Y out of other molecules, also possibly present in the environment and capable of permeating through the membrane, based on molecular recognition.

This is the target of our current theoretical and experimental attempts under the label of the Chemical Autopoietic AI Research Program. Although building an autopoietic synthetic cell from scratch remains the principal purpose, any "intermediate" cell-like structures that can be constructed in the lab are highly relevant. Though not displaying an autopoietic organization, they are per se interesting milestones to reach to contribute to our understanding-by-building undertaking.

The proposal of this SB-AI approach to embodied AI is not driven by the intent of reducing its inquiries to the chemical level. Instead, the goal is to integrate current research approaches that—as enactive AI [82], or organismically inspired robotics [79]—are attempting to implement simplified or abstract forms of the autopoietic organization in robotic systems. That is: our goal is not to substitute their levels of inquiry, but to add and correlate to them a new research level focused on the implementation of the autopoietic (cognitive) organization in minimal chemical models of life. We think that the coordination and mutual enrichment of these levels of inquiry currently represents the only way for embodied AI to shift from a "morphological" to an "organizational" approach and to work on fully realizing the "embodiment turn" in the construction of (mechanical, biochemical or mixed) artificial cognitive systems.

Acknowledgments

The authors thank Pier Luigi Luisi (Roma Tre University and ETH Zürich) for inspiring discussions. This work has been stimulated by our involvement in the European COST Action CM-1304 "*Emergence and Evolution of Complex Chemical Systems*" and TD-1308 "*Origins and evolution of life on Earth and in the Universe (ORIGINS)*."

Appendix

A. A Perspective on Autopoiesis in Four Questions and Answers

A.1 What Is Autopoiesis?

Autopoiesis is a theoretical perspective developed in the 1970s by two neurobiologists, Humberto Maturana and Francisco Varela [85, 86]. The theory intends to provide a scientific answer to the most classical question of biology—What is life?—and, at the same time, to the classical question shared by philosophy of knowledge, philosophy of mind and the cognitive sciences—What is cognition? [87]. The main

points of the autopoietic cognitive biology proposed by Maturana and Varela can be schematically summarized as follows.

- The distinctive property of living systems is their autopoiesis (namely, self-production), that is, their capability of producing and maintaining their material identity (themselves) by producing their own components (via metabolism).
- Since autopoiesis is a global property, its realization does not rely on components of the living systems as taken separately, or specific parts or centers within these systems, but on the way in which the components are organized within living systems.
- In its minimal manifestation, given at the level of minimal cells, the autopoietic organization is a self-regenerating network of operations of synthesis and destruction of components (i.e., metabolism), which: (*i*) produces its material components; (*ii*) defines by itself its topological limits through the creation of a material separation from the external environment (i.e., the cellular boundary); and (*iii*) maintains itself as a unit by compensating environmental perturbations through self-regulation.
- The self-regulative adaptive activity of autopoietic systems can be interpreted as a cognitive activity, consisting in maintaining the structural coupling with the environment by generating internal meanings, expressed in schemes of self-regulation, for external events perceived as perturbations [15, 87].

A.2 In What Sense Is Autopoiesis Related to Embodied Cognitive Science?

Usually the theory of autopoiesis is listed among the most influential groundbreaking theories of the embodied CS, and, in particular, of its more radical expressions—the research lines composing a subdivision of the embodied CS often called "radical embodiment" [4, 88]. The autopoietic framework, schematically summarized earlier, brings forward the radicalness of the autopoeitic approach to the embodiment of cognition. What these assumptions express is the theoretical option of grounding cognition in the processes of self-production of the biological body—more precisely, in the organization of living beings, which sustains these processes. Additionally and coherently, these premises entail a second strong option, that is, individuating the original form of cognition down at the roots of the tree of biological evolution, at the level of minimal living systems—minimal cells.

A.3 How Does Autopoiesis Define Cognition?

By interpreting cognitive processes as dynamics of self-regulation supporting the structural coupling with the environment, autopoiesis reformulates the classical scientific description of cognition, in terms of symbolic information processing, proposed by computationalist

CS. This switch of perspective expresses the refusal of classical representationalism in favor of a "radical constructivism" position. The main differences are schematically summarized in Table A.1.

Aspect of Cognition	Computationalist Description	Autopoietic Description
Interaction of the cognitive system with its environment	Reception of externally predefined information.	Dynamical interference (mutual perturbation) between operationally independent systems.
Cognitive elaboration	Symbolic computation operated on the syntactic aspect of physical symbols.	Stable association of exogenous perturbations to endogenous patterns of self-regulation.
Relationship between external and internal factors in cognitive processes	Internal changes are determined by environmental actions according to an input-output logic.	Internal changes are triggered by external events, but determined by internal processes of self-regulation.
Outcome of cognition	Solution of abstract and externally defined problems based on internal mapping/representation of an external independent reality.	Solution of the intrinsic problem of maintaining the organization in an ever-changing environment based on the creation of endogenous meanings (i.e., self-regulation patterns) for the environmental events perceived as perturbations.
Cognitive relationship (as system-environment adaptive relationship)	Unilateral adaptation of the system to the external conditions.	Structural coupling: a coupled evolution (co-evolution) made of continuous reciprocal perturbations and endogenous compensations.

Table A.1. The computationalist and the autopoietic description of cognition.

A.4 How Can Autopoiesis Guide Experimental Synthetic Biology Research in Embodied Artificial Intelligence?

As we emphasized elsewhere [40], the self-regulative processes performed by autopoietic systems equate to series of changes in the dynamics of their components compensating the external destabilizations that triggered them. As we propose, achieving this in the context of SB can be done by means of bistable genetic circuits (cf. Figure 5). The environment, in the form of physical or chemical factors, can activate the self-regulative transition from an autopoietic state to another, involving the emergence of minimal cognitive operations in the synthetic autopoietic unit.

https://doi.org/10.25088/ComplexSystems.27.3.199

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