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Living cells and biological mechanisms as prototypes for developing chemical artificial intelligence

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ABSTRACT

Artificial Intelligence (AI) is having a revolutionary impact on our societies. It is helping humans in facing the global challenges of this century. Traditionally, AI is developed in software or through neuromorphic engineering in hardware. More recently, a brand-new strategy has been proposed. It is the so-called Chemical AI (CAI), which exploits molecular, supramolecular, and systems chemistry in wetware to mimic human intelligence. In this work, two promising approaches for boosting CAI are described. One regards designing and implementing neural surrogates that can communicate through optical or chemical signals and give rise to networks for computational purposes and to develop micro/nanorobotics. The other approach concerns "bottom-up synthetic cells" that can be exploited for applications in various scenarios, including future nano-medicine. Both topics are presented at a basic level, mainly to inform the broader audience of non-specialists, and so favour the rise of interest in these frontier subjects.

1. Introduction

Artificial Intelligence (AI) and robotics are definitely impacting human societies. They help humans in their mental and manual efforts [1]. They assist or replace humans in specific tasks; sometimes, they can even go beyond human performances. AI promises to automatize some peculiar human capabilities, such as making decisions in environments dominated by uncertainty, partiality and relativity of truth. AI will assist humans in dealing with Complex Systems and facing global challenges [2]. There are, however, also other important problems that originate and reside in the physicochemical domain of living matter and that could be approached in a radically new way, by allowing artificial systems "speaking the same language" of the molecular world. If humans want to provide practical tools to defeat diseases and extend human longevity, improve bioproduction, clean up the environment, and ultimately improve life quality in the long term, they must "colonize" the molecular world, too [3]. The "colonization" of the molecular world becomes feasible if what we call intelligence at all scales (from humans to bacteria) is mimicked through molecular assemblies. The design and implementation of intelligent and autonomous chemical systems are within reach if living cells and biological mechanisms are taken as prototypes. Such a form of AI, which has been called Chemical AI (CAI) [4], has clearly different goals, tools, and methods of conventional AI and is, to date, still at the beginning of its recognition and development. In addition to its potential utility, the study of CAI is, *per se,* interesting from a theoretical viewpoint.

Living beings show a mesmerizing variety of strategies for encoding, collecting, processing, storing, and communicating information. When information is encoded chemically through molecules or atomic ions, its principal transfer mechanisms are diffusion, advection, chemical waves, and through motor proteins. Diffusion is driven by thermal energy or chemical gradients. Physical gradients, such as thermal or momentum ones, drive advection. Chemical waves are autocatalytic reactions propagating through excitable media. Finally, motor proteins exploit chemical energy to move unidirectionally along the railway system of every cell, which is its cytoskeleton. But living beings, made of molecules, can communicate by sending physical signals as well, such as electromagnetic or mechanical waves. This outstanding variety of communication strategies is a wealthy source of inspiration for designing biomimetic physicochemical Information and Communication Technologies (ICTs).

For the full development of such research directions, artificial chemical systems should handle physicochemical information in a

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Abbreviations

AI	Artificial Intelligence
CAI	Chemical Artificial Intelligence
BZ	Belousov-Zhabotinsky
ICT	Information and Communication Technology
IoBNT	Internet of Bio-Nano-Things
MC	Molecular Communication
SC	Synthetic Cell

lifelike manner so that they can be efficiently interfaced with biological counterparts at all scales (organisms, organs, tissues, cells, molecules). For example, the capacity to design systems that process and exchange information in the wetware domain is a prerequisite for implementing a multi-scale artificial/biological communication network, which has been named the "Internet of Nano/Bio-Things" (IoBNT) [5–7]. It essentially refers to a future scenario whereby chemical computation and chemical communication will be fully exploited to construct communicating networks of artificial and biological elements, such as those for monitoring biological functions or healing the body from within. While the realization of the IoBNT is rather speculative at the moment, the field of molecular computation clearly offers interesting research opportunities, a deep understanding of how biosystems function, and genuine scientific progress.

Section 2 of this work presents more details on the motivations and scopes of CAI. Then, two promising approaches for boosting chemical computation and chemical communication are described. One refers to the design and implementation of neural surrogates and their networks, which can communicate through optical or chemical signals for computational purposes and to develop micro/nanorobotics (Section 3). The other approach concerns "bottom-up" Synthetic Cells (SCs) that can be exploited for applications in various scenarios, including future nanomedicine (Section 4). Both topics are presented at a basic level, mainly to inform the broader audience of non-specialists and favour the rise of interest in these frontier subjects.

2. Motivations and scopes of chemical AI

We consider CAI as a broad field encompassing basic and applied research. From the viewpoint of basic research, CAI approaches can lead to theoretical and material models whose development is for investigating aspects of biological intelligence (here we use the term "intelligence" in its wider sense, i.e., as a feature of all organisms). From the viewpoint of applied research, CAI tools can generate novel technologies to solve specific problems.

The need for CAI, intended as a complementary research field to traditional AI, is related to the attempts at "colonization" of the microscopic world, as alleged by Ray Kurzweil in his book *The singularity is near* [3]. CAI is based on a form of computation, embodied as bio/chemical reactions, assemblies, systems, and networks, that can extend conventional computation in the chemical "domain" of fluid mixtures (i.e., wetware systems). The chemical domain of fluid solutions is indeed inaccessible by the symbol-manipulation strategies and algorithms of software-based AI, as well as by the neuromorphic engineering implemented in hardware. CAI should not be seen as a competitor of traditional AI but rather as a "different" AI operating in another domain: the chemical one in fluid environments [8].

The motivations behind chemical computation and CAI, therefore, originate from the consideration that while humans have developed current AI being inspired by human intelligence (specifically, the human rigorous logical thinking) and human brain structure and functions (i.e., the networks of neurons in the brain), the actual implementation of AI has been developed in the software and hardware domains, which can *simulate* the biological processes of interest (chemical, cellular, etc.), but cannot perform them as concrete models. Exploring the possibilities and limitations of chemical computations to generate a chemical version of AI is, first of all, an interesting scientific question *per se*. It challenges us to become fully competent in exploiting the peculiar nature of chemical systems to realize man-made artefacts that reproduce biological intelligence phenomenologically and, more importantly, by exploiting its organizational principles.

CAI can generate new knowledge and new technology. With respect to the generation of new knowledge, the interest in CAI refers to the possibility of constructing wetware models of biological processes in the same domain (the chemical one) in which they actually occur. Thanks to this possibility, CAI can be used to explore basic dynamics and corresponding computational aspects of, e.g., self-production, self-organization, control by hierarchical constraints, autonomy, collective pattern, communication, differentiation, etc. The goal is to discover the emergence of the various types of "intelligence" and "cognition" from the underlying chemical processes – which ideally take place with minimal interventions by the designer. Bottom-up SCs (see Section 4) can be considered a very useful platform for implementing CAI [9]. When SCs are intended as artificial models of biological cells, CAI approaches can help address age-long questions about the origin of life and basic cognition, especially targeting their realization at the minimal complexity level. It should be emphasized that CAI develops knowledge under a "learning by building" perspective, typical of origin-of-life research and synthetic biology. Importantly, the answers this approach provides can either confirm or disprove pre-existing knowledge and paradigms (those that have been used to build the models under scrutiny). Failing to generate the target biological phenomenology or parts of it should be considered as useful guidance for the investigators, as it might shed light on the current lack of knowledge or about wrong organizational models, and in general, will call for alternative paradigms [10].

With respect to the potential spark of new technologies, chemical computation and, therefore, CAI has peculiar features that could allow properly constructed chemical systems: (1) facing some specific computational problems in a unique manner; (2) interacting and co-computing with biological systems. Examples of the first problems are listed below. CAI allows sensing physicochemical signals generated in the microscopic world and communicates them in the same world through diffusion, advection, chemical waves and motor proteins [11] or to the macroscopic world [4,12,13], and extending the sensitivity of the human sensory system [14,15]. CAI allows computing through excitable chemical systems coupled to diffusion, DNA hybridization reactions, networks of biochemical reactions, proteins, chemical oscillators to recognize variable patterns and solve NP-hard problems [16–23], and process vague information [9,24–26].

Examples of the second type can be found in the attempt to interface an artificial chemical system with a biological system. In particular, we can refer to the development of bottom-up SCs (Section 4) whose construction represents, perhaps, one of the most ambitious goals in CAI. Because SCs and biological cells exist in the same domain, they can easily exchange matter, information, and energy. The case of SCs that "communicate" with biological cells aptly shows aspects of the utility of CAI devices for potential applications. In order to control SC behaviour, CAI devices can be introduced in their structure, e.g., signalling networks whose dynamic is similar to feed-forward neural networks or gene regulatory networks whose dynamics are similar to recurrent networks. Despite the fact that using these sorts of biochemical networks for chemical computation and cellular decision-making is not a novelty [27–34], the corresponding experiments are not yet routinely applied in bottom-up SC technology – the subject discussed in this article. Another related scenario, still less explored, refers to communities of SCs, assembled in a tissue-like manner. A possible CAI goal would be the generation of a coordinated behaviour in the SC assembly, based on between-SCs and within-SCs chemical signalling and on the set of

constraints deriving from SC positioning, as well as on the diffusion and reaction rates. Such patterns could mimic elementary steps in differentiation/morphogenesis, although in a rudimentary form. The resulting SC-based CAI could pave the way to the generation of higher-order self-organising structures for biotechnological applications [35] such as "smart" drug delivery and other biomedical applications [36–39], tissue engineering [40–44], biomolecular testing, regenerative processes [45, 46].

In the same way conventional AI aims at constructing hard and soft robots, CAI aims at constructing their counterparts in the fluid chemical domain, i.e., a sort of "Chemical Robotics" [47–49]. Chemical robots offer remarkable promises as far as versatility, adaptability, resiliency, distributed architecture, and autonomy are concerned. A key difference between conventional robotics and chemical robotics is immediately evident. The fluid chemical domain provides the opportunity for a computational paradigm that deviates from the well-known von Neumann architecture. In particular, what is computed (the data) and what makes the computation (the computer) are both in the same category, have the same physical nature, and can exchange their role. The identity and the structure of chemical species, the interactions they generate, their resulting concentrations, and the magnitude of their reaction rates are all intimately connected. CAI systems can be analyzed for their capability of processing information according to the usual syntactic perspective (e.g., symbol manipulation, logic gates, memory, …). In most cases, this is a convenient and adequate way of looking at CAI systems, especially when the focus is not the system itself, but the transformations it generates from its input to output signals. However – and this is a key peculiarity of CAI – it is also possible to look at CAI systems and their functioning from a genuine self-emerging semantic viewpoint. In particular, this is possible because when the existence of a dynamical chemical system depends on autonomously evolved selections of the environmental signals (it picks up and accommodates into its own dynamics, without falling apart) a meaning becomes autonomously and intrinsically associated to those signals: favourable or detrimental for the system existence (persistence) as a dynamical entity.

3. Neuromorphic engineering in wetware

Some performances of cells, such as self-replication, metabolism, adaptability, and information handling, can be imitated through molecular assemblies, whose chemistry is entirely different from that of natural cells but such that they constantly work in out-of-equilibrium conditions as biological cells do [50]. When the research focuses on the design of chemical ICTs, the idea of devising neural surrogates and artificial neural networks is particularly alluring. One of the purposes of neuromorphic engineering is to develop brain-like computing machines revolutionising von Neumann's architecture of current electronic computers. Electronic computers are digital and reliable. The operations are executed with a precise frequency, and binary digits cannot be misinterpreted because they are associated with low and high voltage values of transistors. The race towards an always faster computing rate has been accomplished through constant miniaturization of the transistors. We are reaching a limit because transistors are constituted of a few atoms, and the very fast computational rates come at the price of an unsustainable energy cost. A valuable alternative is a brain-like computing machine. It is expected that a brain-like computing machine will manifest those computational performances that are peculiar to the human nervous system. It will be both digital and analog: the

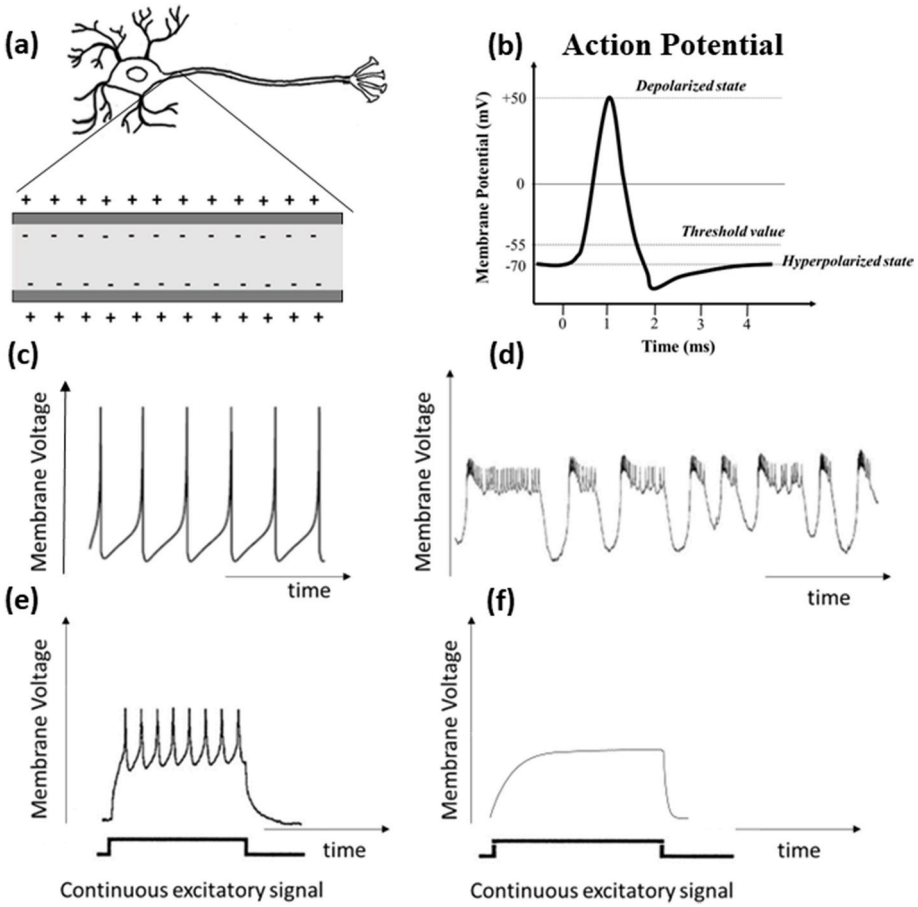

Fig. 1. Magnification of a neural axon's portion showing its transmembrane electric potential in (a); profile of an action potential in (b); electrical responses of neurons in oscillatory (c), chaotic (d), tonic excitable (e), and phasic excitable (f) regimes.

information will be encoded in a variable reproducing the features of the transmembrane electric potential in real neurons, which changes in an analog manner, yet spikes can be counted (see Fig. 1). Due to their mixed nature, neurons combine adaptation and reliability. They inherit the adaptation of analog systems in their subthreshold regime, but the timing of their discrete events inherits the reliability of digital circuits and automata [51]. Furthermore, a brain-like computing machine will speed up computations through the parallelism of neural networks; it will have its processor and memory in the same place, not physically separated like in current electronic computers. The presence of recurrent networks will guarantee the capacity for learning from data and speed-accuracy trade-offs [52]. Finally, a brain-like computing machine will reproduce the human capability of making decisions using natural language because it will be capable of processing Fuzzy logic [53,54]. Fuzzy Logic Systems process both analog and discrete information since they handle both words and numbers. They show adaptability and reliability, as was demonstrated by fuzzy controller hardware systems to (1) stabilise an inverted pendulum, (2) control a space booster rocket and satellite, an automatic aircraft landing system, (3) model and control chaotic systems, and other applications which need swift approximate reasoning [55–58].

3.1. Neurons and neural networks

In animals, neural networks are specialized in collecting, memorizing, processing, and sending information. These essential functions confer to living animals the capability to pursue goals such as surviving and reproducing. Every individual building block of the nervous system, i.e., the single nerve cell, is specialized in encoding, collecting, processing, and sending information. At the cellular level, information is encoded in the value of the neural transmembrane potential (see Fig. 1a). When it is negative, the neuron is in the so-called hyperpolarized state. On the other hand, when it is positive, the neuron is in its depolarized state. Signals reaching a neuron determine transmembrane potential changes. If the signal is inhibitory, it hyperpolarizes the neuron. If it is sufficiently activatory, it depolarizes the neuron. The neuron can respond by firing an action potential (see Fig. 1b), which is a quick transient change (occurring in a few ms) of the potential from negative to positive and finally to original negative values. The action potential propagates through the neuronal axon as an electrochemical wave. It reaches the bottom part of the neuron, which is made of synapses. Synapses respond to the wave by releasing chemical signals to other network neurons. The computational capabilities of every nerve cell depend on the number and types of synaptic connections established with other neurons and on its intrinsic dynamical properties. Every neuron is a nonlinear dynamical system [59,60] that can work in different dynamical regimes. It can be a (1) pacemaker neuron when it fires action potentials periodically (see Fig. 1c). It is in a (2) chaotic regime when it fires action potentials aperiodically (see Fig. 1d) and is extremely sensitive to external stimuli. It is (3) an excitable neuron if it stays indefinitely in its resting state without firing action potentials unless it receives excitatory signals. If this is the case, it can respond by firing a train of action potentials (in this case, it is (3a) a tonic excitable neuron as shown in Fig. 1e) or in an analog manner, with a transmembrane potential change proportional to the extent of the stimuli (in this case, it is (3b) a phasic excitable neuron as shown in Fig. 1f). The communication among neurons determines their synchronization. At the network level, information is encoded through the spatiotemporal synchronization phenomena that can emerge within the neural web. This is the reason why the human brain has been defined as a "Time--machine" [61], and cognition has been interpreted as the global integration of local neuronal operations [62].

3.2. Neural surrogates in wetware

Artificial neural networks can be implemented in wetware by reproducing the dynamics of real neurons through proper chemical systems and putting such neural surrogates in communication through electro-chemical or optical signals [63–68]. Special reactive chemical systems can reproduce the dynamics of oscillatory, chaotic, and tonic excitable neurons. One example is the well-known Belousov-Zhabotinsky (BZ) reaction, which is an oxidative bromination of malonic acid in an aqueous acidic solution [69]. Its dynamical behavior depends on some physicochemical conditions, such as the concentrations of the reagents. Due to its irreversibility, the BZ reaction can work indefinitely in time only if carried out in an open reactor because the reagents progressively run out. This technical drawback can be overcome by designing neural surrogates through photochromic and luminescent materials [70]. The absorption of UV–visible radiation by photochromic and luminescent compounds induces a reversible color change and light emission, respectively. Single photochromic and luminescent compounds have been proposed as surrogates of phasic excitable neurons [71]. When their optical response is coupled to a convective motion of the solvent, their solution can reproduce the dynamics of chaotic neurons [72]. Finally, oscillatory photochemical systems have been designed by combining pairs of properly chosen photochromic compounds [73,74]. They have the relevant asset of working indefinitely in time within closed reactors. Furthermore, they can communicate through optical signals and give rise to an opto-based IoBNT, which can easily interplay with the Internet cyber-domain based on electrical circuits and electromagnetic communications [5,6]. Neural surrogates that exchange optical signals can synchronize likewise to what occurs in natural feedforward and recurrent neural networks [75]. When synchronization involves chaotic signals, it allows secure communications and encryption [71,76–78]. The wavelength-dependent response of photochromic compounds makes them appropriate for reproducing neuromodulation [79]. Neuromodulation is the power of reconfiguring neural networks into different functional circuits depending on the stimuli, allowing animals to learn [80].

3.3. Opto-based IoBNT

Opto-based IoBNT, reproducing neural networks, can be exploited for unconventional computing. In such opto-based IoBNT, information can be primarily represented by frequencies and phases of oscillatory signals, and the interactions of the elementary neural surrogates drive the internet into collective states that are the results of the computations [81]. Such collective states are attractors of nonlinear dynamical systems. Attractors can be fixed points, limit cycles, and chaotic trajectories in phase space [82]. Often, a multitude of accessible attractors coexist, and the overall dynamical system is said to be multistable [83]. Opto-based IoBNT may be valuable for facing pattern recognition problems and computationally hard problems. When the IoBNT simulates a recurrent network with many elements interconnected through feedback actions, it can be used to implement Reservoir Computing. In Reservoir Computing, an input pattern is transformed into high-dimensional spatiotemporal patterns of the recurrent network so that a simple learning algorithm can efficiently read out the input features [84]. On the other hand, when IoBNT allows the implementation of Oscillation-Based Computing, it can become promising for facing large NP problems that are notoriously intractable by conventional electronic computers [81]. The computational performances of IoBNT will depend on the number of elementary computing elements. A large number of computing bricks can be stored in a relatively small volume only after miniaturizing every single neural surrogate. Instead of mechanically shrinking macro-reactors (like single cuvettes), it looks promising to implement the building blocks of IoBNT in single droplets of microemulsions [85], liposomes [86], liquid marbles [87], and nanocapsules [88]. Extreme miniaturization can be achieved when single proteins are exploited as neural surrogates.

3.4. Proteins as neural surrogates

Proteins are the fundamental ingredients of the signaling networks of every cell. The signaling network is a web of proteins that plays the role of the brain in animals [89]. The proteins of the signaling network have the collection, transduction, transfer and processing of information as their primary functions [90]. These proteins give rise to a highly intertwined recurrent network through nonlinear allosteric and other interactions. The information-handling power of every protein depends on its three-dimensional structure. Initially, it was thought every protein has a well-defined and rigid 3D arrangement of its atoms. This idea promoted the formulation of the strongly selective lock-and-key paradigm for the description of protein-and-substrate interplay [91]. It is now recognized that many proteins or some of their portions are structurally disordered in their native, functional states [92]. They are conformationally dynamic and exhibit functional promiscuity. They can show context-specific functions in response to changing environmental conditions, and a single structural motif can be used in multiple settings [93]. All these features make proteins appropriate for processing fuzzy logic [94,95]. Actually, all the principal macromolecules that are within living cells, proteins, RNA, and DNA exist as collections of many conformers. Since conformational distributions work as molecular fuzzy sets [25,26], it can be inferred that the logic of life is fuzzy, i.e., vague. The homeostasis and purposefulness of living organisms depend upon a network of regulatory mechanisms, i.e., negative feedback loops [96,97]. Such loops involve proteins, RNA, and DNA molecules. Therefore, these regulatory networks are intrinsically fuzzy. Their fuzziness guarantees adaptability and the capability to make decisions in environments dominated by uncertainty and vagueness [24,98]. These performances can be extended to networks of properly chosen proteins [99] or other macromolecules and hence IoBNT, where communication is mainly based on chemical messages.

4. Bottom-up synthetic cells

The name Synthetic Cells (SCs), with the prefix "bottom-up", refers to micrometric or submicrometric cell-like structures fabricated from scratch by processes of self- and guided-assembly of molecular components of various sorts. This research is now recognized as a part of synthetic biology but the very idea of constructing cell-like structures (and, ideally, synthetic living cells) is quite old. Its roots date back to the beginning of origin-of-life studies [100], and more recently to experiments on chemical autopoiesis in the early 1990s [101–103]. According to the understanding-by-building paradigm, bottom-up SCs have the potentiality of playing important roles in basic and applied science. In basic science, SC research can be directed toward addressing questions like "what is the minimal complexity of cellular life?", or "when do inanimate systems become alive?", or "can chemical systems display cognitive features?". In applied science, properly designed SCs can become innovative and versatile biotechnological tools for assays, biosensing, lab-on-chip and microfluidic devices, and in nanomedicine as "smart" drug-delivery or drug-producing agents [36,104]. In this second context, it can be convenient to conceive SCs as wetware machines, and thus interpreting their functioning from a computational perspective. Hence, machine-like SCs can be designed, realized and analyzed from a bio/chem-ICTs viewpoint, namely focusing on the associated information and communication processes that take place in these molecular systems. Applying the concepts of information and communication to SCs behavior can be indeed useful for a more quantitative description of their dynamics, making a step beyond the usual qualitative description (e.g., SCs have been, or have been not, able to perform certain actions). Indeed, in this applied science perspective, SCs are designed and built with pre-fixed goals in mind, they are conceived as biotechnological tools, and more often than not they need to "do something" (e.g., complete a task) that is not necessarily related to their own self-maintenance (even if self-maintenance-like processes can be functional to achieve their goals). When systems display these features, they are *allopoietic*, meaning that they produce something "other" than themselves, and rather resemble machines instead of organisms (the term allopoietic is used in opposition to autopoietic, or self-producing, introduced by Maturana and Varela to describe and characterize any living systems [105]). There are many opportunities for designing and constructing allopoietic SCs. Although they can still share some extremely simple traits of biological cells (self-bounding, pieces of metabolic networks, transcriptional control, signaling, etc.), the important constraint of all component self-production (autopoiesis) is dropped off. In this context, SCs can be "programmed" by the designer to accomplish some specific tasks, e.g., produce compounds with pharmacological activity (to be released in the environment and, e.g., heal or kill a sick biological cell), or act as a machine to manipulate chemical information. It also follows that control mechanisms in allopoietic SCs are easier to achieve when compared to autopoietic systems.

The following sections will be dedicated to briefly survey concepts and technologies behind bottom-up SCs, mainly from the viewpoint of a chemical computational perspective – the theme of this article. SCs will be described as wetware machine-like allopoietic systems that process chemical information. From this perspective, they need to be equipped with a sort of CAI to perceive, actuate, control.[1]

4.1. A bottom-up SCs primer

As mentioned, bottom-up SCs are artificial solute-filled microcompartments whose typical size range from 0.1 to 10–20 μm. They can be considered cell-like systems, because the reactions that take place in the SCs make them resemble, in a partial and rudimentary way, biological cells. But no living SCs have been constructed to date. SCs can be prepared by encapsulating selected molecules – typically, but not necessarily, biological (macro)molecules – inside microcompartments like lipid vesicles (liposomes). Other compartments can be also used, such as fatty acid vesicles, polymer vesicles (polymersomes), coacervates, water-in-oil emulsion droplets, water-in-oil-in-water double emulsion droplets, particles formed via liquid-liquid phase separation, etc. The liposome model, however, seems to best imitate biological cells as it provides an aqueous lumen that hosts water-soluble compounds and a semi-permeable membrane (capable of hosting membrane proteins too) in a very biomimetic and cell-like fashion.

Here we would like to concisely introduce this subject by means of selected Q&A, typically made by those who are less familiar with the topic. Several excellent and extensive reviews should be consulted, instead, for more information [109–121].

How are SCs actually made? What are the enabling technologies? How long are SCs stable? There are several techniques to build SCs, very much depending on the actual system one plans to build. In the widespread case of liposome-based SCs, liposomes are allowed to form in an aqueous solution that contains the solutes of interest, i.e., those that must be encapsulated in the liposome lumen. Lipids spontaneously form a membrane, via self-assembly, and the membrane eventually closes to form a microcompartment. Many solute molecules will not be

[1] It should be noted that aside the computational perspective, and especially when SCs are intended as model of biological cells (i.e., organism-like autopoietic systems), the "computer paradigm" can be substituted with alternative views, inspired to self-organization and autonomy, and involving concepts such as structural coupling, enaction, affordance, *Umwelt*. This second perspective has the potential of developing original SC-based research lines across the fields of Artificial Life, Cognitive Sciences, and ultimately explore new forms of AI. CAI, in this second sense, has different connotations and faces different questions, often more related to basic science, such as minimal cognition, emergence of meaning, and mind-likeness. A discussion about these aspects lies outside the scope of the present article, and has only recently begun to be considered as a topic in SC research [106–108].

encapsulated and need to be removed or inactivated. Current research is based on the convergence of liposome technology, biophysics, microfluidics and micromanipulation, in vitro biochemical reconstitution and cell-free gene expression. Deterministic and stochastic numerical modeling can be employed to help design or analysis of SCs. Because a variety of efficient techniques are available for the construction of various types of SCs, especially in the case of liposomes, it can be said that the encapsulation "problem" is under control. Difficulties may arise in the case of functionalization with membrane proteins, or when the small compartment size challenges the statistics of co-encapsulation of several chemical species [122,123]. In particular, the droplet transfer method should be mentioned as an efficient and convenient method for constructing SCs based on giant lipid vesicles [124–126]. The design of internal reaction networks is based on what biochemical mechanisms need to be reconstructed. In vitro gene expression, transcription regulation, signaling, multi-step enzyme-catalyzed metabolic pathways, and mechanisms acting on the membrane are among the most well-studied. The functioning and the persistence of SC components (especially macromolecules or complexes like the ribosomes) determine the functional "stability" of SCs. The stability of macromolecular 3D structure, which determines their functioning, can be hampered or jeopardized by the plethora of possible chemical interactions (with other macromolecules, with unwanted small molecules that accumulate in the SC, with ions including proton and hydroxyde, i.e., pH, with lipids and other amphiphiles), and by temperature. As any other colloidal system, SCs integrity and physical stability against aggregation, fusion, and dissolution are dominated by surface forces. Depending on their structure and storage conditions, SCs functioning and stability can range from hours to days.

What is the state-of-the-art of SCs research? Are SCs alive? SC research is conceived as a multidisciplinary arena that attracts scientists from different disciplines (biophysics, chemistry, biology, engineering). This unique multidisciplinary platform generates a vast array of experimental studies facing disparate questions: from the origin of life to the reconstruction of complex cytomimetic mechanisms, from the recognition of minimal complexity [127] to the mimicking of cellular communication. Recent studies highlight the potential applications of SCs as drug-producing devices [37]. Literature analysis shows that several milestones have already been reached; it is often possible to carry out complex biomolecular processes in SCs. But the seamless integration of different independently-optimized "modules" is one of the next challenges [128]. Despite the recent indisputable advances, no living SCs have been constructed so far. SCs steadily working out of equilibrium is another relevant target [129]. Considering the energy issues, putting aside the few and relevant examples of ATP-producing SCs [130–132], most of the reported SCs just function as "wind-up toys". They consume an initial cargo of energy sliding on the descending branch of a free energy profile.

Which theoretical frameworks generally apply to SCs? SCs were originally intended as tools to demonstrate the emergence of (minimal) life from inanimate matter – in an origins-of-life perspective (even if built with "modern" molecules such as enzymes, ribosomes, etc.) [133–135]. To date, SCs are instead intended under a broader perspective, not necessarily linked to primitive systems, but also looking at biotechnological applications. It follows that different theoretical frameworks can be applied to the SC field, depending on the scopes. Most of the SC research practitioners interested in basic scientific questions explicitly refer to autopoiesis [105] and chemoton [136] theories. When aspects of regulation and control are discussed, the dynamics of chemical networks in SCs should be conceived in order to reproduce two relevant self-regulation mechanisms of living cells, such as homeostatic (reactive) and allostatic (predictive) ones [137]. Homeostasis requires negative feedback mechanisms and allows to maintain physicochemical steady conditions in a changing environment. On the other hand, allostasis involves a feed-forward mechanism and allows a continuous re-evaluation of needs and readjustment of all parameters toward new set points in response to external changes. Allostasis ("stability through change" [137,138], as opposite to the "stability through constancy" of Bernard-Cannon homeostasis), was introduced to overcome limitations of conventional homeostatic control theory, considered as overly restrictive and reactive in character. Allostatic regulation, instead, is based on prediction (anticipation) as it requires a continual preparation for what might be coming next. Allostasis differs from homeostasis in its predictive character and in its ability to anticipate and adapt to change rather than resist it [139]. As mentioned at the beginning of Section 4, the mathematical language of information and communication theory can be applied to SCs intended as machines that process information in the chemical domain. To date, SCs are often very simple and such an analysis is generally not carried out, but future developments can lead to more complex SCs whose behavior might be advantageously described by these methods. Another interesting aspect stems from considering the construction of SCs as the wetware branch of Artificial Life, beside robotics and AI. Seen from such a viewpoint, investigating bottom-up SCs falls into the epistemological domain of the "sciences of the artificial" [140], i.e., those sciences that investigate natural processes through the constructive/synthetic method (explaining = constructing, [10]). When SCs are intended as biotechnological tools, however, classical theoretical frameworks referring to the first cybernetics and control theory, as well as information and communication theories can be usefully adopted to explore and interpret these novel wetware systems. In this article we will mainly discuss this second perspective.

SCs: Quo vaditis? SCs research is continuously progressing and it will move forward in important directions, such as: (a) standardization of the "modules" developed until now, aiming at their seamless integration to build more complex functions; (b) construction of SCs with nested design (e.g., vesicles inside vesicles, the internal compartments intended as artificial organelle); for the compartmentalization of otherwise incompatible "modules" and for exploiting transmembrane vectoriality and chemical gradients; (c) focusing on generating systems in a steady out-of-equilibrium state, emphasizing the open-flow reactor nature of SCs; (d) advancements in robust and efficient ATP-producing mechanisms; (e) development of a whole-SC core-and-shell self-reproduction, i.e., being able to integrate biochemical production processes with whole-system biophysical and biomechanical path for grow-and-division; (f) shift from studies on isolated SCs to communities of SCs and tissue-like systems, reaching higher levels of integration, coordination, synchronization based on physico-chemical communication mechanisms; (g) with respect to chemical communication, improvement of robust, orthogonal, and space-time controlled information processing mechanisms (see Section 4.2). The posed challenges, and at the same time the rewarding gains that SC research is promising, are therefore very exciting. The next section, which resonates well with the topic of the present article, is dedicated to the last subject in the list.

4.2. Communication and information handling mechanisms in SCs

Chemical computation and chemical communication capabilities are always crucial in any scenario involving SCs. When applied research is considered, it is possible to imagine SCs that have been designed not to investigate the basic organizational (autopoietic) principles of life, but to carry out tasks relying on chemical computation, control, and decision making. As anticipated at the beginning of Section 4, in this context SCs can be conceived as allopoietic machine-like systems with goals (pre-fixed by their designer and useful to her), which do not coincide with self-production, self-distinction, self-maintenance, but aims at something else. For example, SCs can be imagined as traveling agents that move in a blood capillary, targeting sick cells. The aim could be to heal or to kill them by producing and releasing a therapeutic compound. Or, while employing SCs for a complicated bioassay, one can imagine properly designing SCs in a microfluidic device or in microwells. These SCs, endowed with protein receptors on their membrane and *ad hoc* reconstructed intra-SC signaling networks, could be used for screening

huge chemical libraries, searching for specific matches. In another foreseeable setup, SCs could be precisely placed in 3D structures made of biological cells, aiming at directing or at least influencing growth and differentiation processes. In all examples, well-known concepts of communication and information handling apply, but they need to be properly adapted to the specific material that supports these processes, namely, molecules and wetware microenvironments.

Various types of SCs, even if they differ in complexity, essentially consist in compartmentalized chemical networks and consequently they are endowed with a boundary that generates a distinction between SCs and their environment. SCs are then conceived as systems, endowed with programmable internal mechanisms, exposed to an external world that affects SC behavior via physico-chemical interactions. The environment can be a fluid solution or a gel in which chemicals have been dissolved, or can be made of other SCs or biological cells that produce, send, and receive chemical signals. The concept of chemical computation, therefore, can be related to the chemical transformations occurring in the set of SC reactions. Communication, instead, can be intended in two manners. The first is the common use of the word made in experimental research, e.g., a SC that communicates with a biological cell by exchanging chemical signals. The second is more theoretical, and is about interpreting the dynamics of a chemical system (a reaction network, a sensor-actuator module, the entire SC) as an information channel, *à la* Shannon, as a computing machine that transforms input signals to output signals [127].

Searching references about chemical computation and communication in the literature of SCs returns case studies in experimental approaches to communicating SCs. Synthetic biologists working on bottom-up SCs already focused their interest in chemical communication as early as in 2009, when Ben Davis and collaborators [141] firstly reported on very simple SCs capable of producing molecules similar to the autoinducer-2 (a furanosyl borate diester functioning as a signaling molecules in quorum sensing), and activate a response in closely located *Vibrio harveyi* bacteria. Following the same principles, but employing biochemical networks based on gene expression, more work has followed [142–144], up to the achievement of a bidirectional SC/biological cell communication, again by means of quorum sensing molecules [145]. Since then, the scope of communicating SCs has been widened and several examples are now available, e.g. Refs. [146–153]. These developments enrich the toolbox of SC practitioners and are the prerequisite for constructing more advanced systems. However, it must be said that in these reports the results are discussed from a purely empirical viewpoint, i.e., describing whether or not the systems were able to communicate, limiting the answer to a qualitative yes/no outcome.

On the other hand, approaches based on Shannon information and communication theory, mathematical modeling and simulation of molecular diffusion, communication networks to chemical communications have been recently developed by the community of communication engineers – independently from the above-mentioned synthetic biology studies. In particular, pioneering reports [11,154,155] have been functional to the development of a rather active community of engineers interested in exploiting molecular communication (MC) as a form of unconventional communication [156], also in reference to the ambitious target of the above-mentioned IoBNT [5–7]. The central theme of MC is the extension of the information-theoretical treatments to chemical communication, mainly looking a bioengineering perspective. MC deals with messages in the form of spatio-temporal patterns of molecules. It identifies several important differences between conventional and molecular communications, ranging from transmission speed, energy requirements, distance covered, accuracy, directionality, and develops mathematical models that soon become very involute, because stochastic descriptions are often required. The possibility of intersecting these two words (synthetic biology and communication engineering) represents a huge opportunity for advancing SC technology and approaching chemical communication in a more quantitative manner.

Based on these premises, some interesting directions for future research can be suggested. Firstly, information theory is based on signals and their probabilistic distributions. Such a kind of "probabilistic thinking" is rarely applied in current experiments dealing with computation and communication between and within SCs (most of the experiments focus on the demonstration that the processes of interest occurred or not). Efforts to look again at already available data and search for probability distributions will be rewarded by the acquisition of a new language for applying ICTs methods and tools, and possibly providing more quantitative descriptions.

The first question is about defining better how information theory concepts apply to SCs (and in general, to chemical systems) and their behavior. The concepts of "states", for example, need to be defined operatively, maybe based on arrays of concentrations and/or fluxes. Consequently, clarify whether or not it is possible to identify regions in the phase space corresponding to certain behaviors. The information-theoretical concept of "channel" can refer to the SC itself, or parts of it, allowing a variety of modelization. For example, two complementary questions arise: (a) what is the optimal distribution of source signals to be transmitted in a given channel, and (b) what is the optimal channel structure (set of reactions) that can transmit a given source signal?

Another example comes from the attempts of measuring "semantic" information associated with a "situated SC" scenario [157,158]. Following Kolchinsky and Wolpert [159], semantic information is intended as the fraction of syntactic information that is causally necessary to an agent to survive, and can be calculated from information-theoretical quantities. The current model is based on a hypothetical SC which responds to a signal molecule, producing a toxin. But more complex situations can be imagined and possibly realized experimentally. Thanks to their low complexity and knowledge of internal mechanisms, SCs could become useful platforms for allowing data-driven analysis and simulations that are otherwise out of reach when applied to natural systems.

Additional inspiration comes from AI. It is well known that biological systems, particularly neurons, have decisively inspired AI, since its origin. Synthetic biology offers another unique opportunity: constructing CAI systems with (bio)chemical parts. As we have commented elsewhere [8,99,160], the point is not only just mirroring AI devices (e.g., neural networks) by employing molecules but also exploiting properties unique to the chemical domain. Parallel computing is a well-known property. Moreover, thanks to the fact that macromolecules not only catalyze reactions or act as receptors, but are themselves the product of other reactions, in chemical networks, the difference between the "computer" and "computed" elements is somehow blurred. Allosteric regulation adds significantly to the possibility of having chemical networks with behaviors, which are sought to achieve specific dynamics.

The construction of chemical neural networks is a fascinating topic. At the moment this issue is still speculative, but it is possible to imagine SCs hosting senso-regulatory mechanisms that control the SC operations in a neural network-like manner [9,99,161–164]. In other words, this is equivalent to implanting a sort of minimal "brain" inside a SC. Going one level up, an SC could be itself a node in a neural network made of other SCs (or biological cells) – imitating a tissue-like system of communicating SCs. To date, both perspectives seem not so easy to implement experimentally, but their dynamics can be explored via simulations. The goal, here, is to discover whether and at what extent the peculiar mechanism of information handling and transmission, typical of (bio) chemical transformation, can be used to drive SC behavior in a certain environment, and cope with uncertainty. In a previous work we have referred to the intrinsic *fuzziness* of these systems [9,24–26,94]. In a perspective of chemical embodiment, it must be considered that the chemical neural network elements are proteins and thus can display conformational diversity. This brings about a shift from a dichotomic two-state (yes/no) logic, as specified by sentences like "the sensor (or the response regulator) is/is not activated", to the continuum case (gray-scale), typical of fuzzy logic.

5. Concluding remarks and perspectives

The development of methods and strategies for CAI appears to be a relevant target for contemporary research in a multidisciplinary territory across chemistry, synthetic biology, biophysics, the sciences of the artificial, and bioengineering. By taking inspiration from living cells and biological mechanisms, CAI is indeed a new form of AI because it is realized not in the symbolic/logical domain (although it can still be modelled according to it), but it is firmly rooted in the physical, material nature of chemical molecules, in their interactions, and their transformations. It is a form of embodied AI that is made possible by mechanisms occurring at the nanoscale, where different forms of energy have similar magnitudes [165,166].

In this work, we have just scratched the surface of a wide and possibly very fertile research arena that can be recognized as the study of what chemistry can offer to the traditional field of AI – to generate a genuinely novel approach inspired to biological organization. Here we have presented two topics that, in our opinion, will be of central interest to CAI. One refers to the attempt to design and implement neural surrogates through proteins and nonlinear chemical systems. Such neural surrogates implemented in wetware (i.e., in fluid solutions) can communicate through physicochemical signals: They give rise to spontaneous phenomena of spatiotemporal synchronization, analogous to those occurring in real neural networks. Human reasoning will be within reach if the structural and functional complexity of the human brain is approached through chemistry in wetware.

The second topic refers to SCs: Even if they still are rather simple structures when compared to biological cells, they can soon become a unique platform for exploring CAI modules in a cytomimetic environment. Goals can range from the control of SC behavior to the recognition of patterns of environmental signals, or from mimicking Bayesian inference through molecular mechanisms to the production of collective dynamics in artificial cellular assemblies.

As mentioned, this article aims to focus on systems, based on chemistry and biochemistry, that can spark interest in the community and stimulate further research and reflection. In these concluding remarks and perspectives we briefly add further considerations related to the topics mentioned above, mainly focusing on the potential of SC research, its theoretical framework and implementations.

In previous sections, indeed, we have privileged for simplicity the machine-like view of SCs and their operations, evoking the analogy between SCs and robotics (SCs understood as chemical robots), and CAI as the chemical implementation of (traditional) AI. In doing so, it might seem that we envisage a path for the development of these new concepts that parallels the historical development of AI and robotics. In reality, although these traditional approaches may be convenient for some objectives, in no case should the CAI be bound to them. For example, the dual scope of SC research actually offers several opportunities both in the field of bioengineering and to explore biology from an unprecedented point of view. The implementation of CAI by SCs, for example, can be seen as a way to build a programmable chemical system with a certain degree of autonomy. There will be a spectrum of behaviour and applications. In the construction of SCs as chemical robots to perform useful actions (e.g., bioreactors, drug delivery agents, etc.), CAI devices can be interpreted within the input-output, instructive, simple machine-like framework. The latter seems, at least to us, adequate for such conceptually simple tasks. In this context, SCs can be seen as programmable robots that sense their environment and operate consequently. But looking at CAI as the "intelligence" of living beings, and considering the different scales and the different types of problems organisms face to survive (in autopoietic language: to maintain, perpetrate, and adapt their organization) makes this theme essentially coinciding with a path toward Artificial Life (ALife). Such a path is the wetware one, the only one that can generate the biological phenomenology, because it is placed in the same chemical domain as life.

Under this more encompassing viewpoint, developing CAI systems becomes a research platform to investigate the nature of living cells through the understanding-by-building approach. It raises fundamental inquiries such as the true nature of SCs, the feasibility of creating a living system with minimal complexity, and the dynamics required to produce a cognitive agent. These questions encourage a dynamic systems approach to comprehend biological systems. Concepts discussed in this article, like differentiating autopoiesis from allopoiesis and recognizing homeostasis and allostasis as important mechanisms for stability, are vital for grasping the chemical intelligence that living systems exhibit. This intelligence is evident in their renowned problem-solving abilities across a vast range of space-time scales and domains, including metabolism, morphology, activity coordination, movement, biorhythms, multicellular development, and even mental operations. Efforts to construct SCs serve as a guide for a deeper understanding of how living organisms maintain a balance between autocatalysis and feedback, or adapt to new conditions through plastic reconfigurations of their dynamic chemical networks. To date, experimental goals are limited to basic SCs. However, initiating discussions on these topics and integrating such concepts into the SC research lexicon will likely inspire future research dedicated to these intriguing subjects.

In the same manner, we support a reinforced role of theoretical biology and dynamical systems concepts for CAI, our mention to Shannon ICT aims at promoting the benefits, especially for bioengineering applications, of more quantitative description of what systems like SCs can do, for instance in the context of molecular communications. The long-discussed (and somehow controversial) theme of information and its significance in biological processes is a topic that, until recently, was not present in SC research [167]. However, in Section 4.2 we have presented a recent approach dealing with "semantic information" that employs information theoretical quantities applicable to SCs. These developments suggest that constructing CAI systems (as SCs are) is a productive endeavor. They can demonstrate the potential for incorporating classical ICT in cell-like functioning, yet acknowledging both the benefits and limitations. It will be possible to explore novel viewpoints and perspectives by means of a previously unattainable experimental platform.

Finally, it should be recalled that although we have mainly mentioned well-known chemical computation systems based on biomolecules, such as phosphorylation cascades (as feed-forward chemical neuron networks) and gene regulatory networks (as recurrent chemical neuron networks), the element of novelty we highlight here are not these systems in themselves. Rather, we highlighted that when current research on SCs is considered, realistic implementations involve engrafting "CAI modules" inside SCs, as a first and doable step on the route to control SC behavior with explicit reference to AI concepts. By starting looking at these chemical systems from under the CAI lens will be advantageous for practical developments and for setting the stage of the first programmable cell-like systems for potential real-world applications.

It should be also recalled that the inherent characteristics of the reactants – and thus their reactions – in a chemical network permit or preclude the formation of specific topologies and computational processes. For instance, enzymes exert powerful selection on the structures they can process as substrates or on substances that may act as allosteric regulators. Conversely, simpler chemical substances not capable of generating such selective constraints must be also considered for developing CAI. This highlights the potential role of "abiotic" chemical systems in CAI.

As stated in the section dedicated to neuromorphic engineering, some performances of neural cells can be imitated through abiotic systems, whose chemistry is entirely different from that of real neurons but such that they constantly work in out-of-equilibrium conditions. Such conditions allow to reproduce the dynamical behavior of real neurons and hence approach some elementary functions of neural networks when the single neural surrogates communicate and give rise to spontaneous synchronization phenomena. Networks of neural surrogates are

valuable in unconventional computing: they can be exploited for facing pattern recognition and computationally hard problems. Information is encoded in the frequencies and phases of oscillatory signals, and the interactions of the elementary neural surrogates drive the network into collective states. Such collective states are attractors of nonlinear dynamical systems: they represent the results of computations.

SC research extends to abiotic systems too, as alluded in Section 4. Specifically, SCs can be built as models of primitive cells (protocells). Several hypotheses have been proposed regarding the nature of abiotic (i.e., prebiotic) chemical systems that may have catalyzed the initial stages of life's chemical origins, particularly the emergence of early cells. When molecular systems made of species with similar functional groups are considered, their reactivity is expected to be more promiscuous, giving rise to intricate reaction networks. In this context, the sort of "intelligence" such complex chemical systems and networks must achieve is "fundamental", straightly connected to capacities such as of self-bounding, self-maintenance, self-reproduction, self-replication. Prebiotic chemistry investigations focus on "systemic" properties of networks, encompassing a wide array of chemical phenomena. These include autocatalytic reactions (e.g., the formose reaction; see Refs. [168–170] for chemical computation aspects), hypercycles, oligopeptides or oligonucleotides self-replication, out-of-equilibrium combinatorial reactions based on labile covalent bonds, reaction-diffusion systems, vesicle and micelle self-reproduction. "Systems Chemistry" is the discipline that explores these topics. Inquiring about the computation capabilities and the CAI potentialities in systems chemistry implies new open questions and experimental/theoretical approaches to understand and later exploit the fundamental chemistry of life. Viewed through this lens, CAI can go beyond the mere imitation of biological intelligence (imitation of performances) and, therefore, be just a "chemical version" of conventional AI. CAI has the potential to generate forms of AI strongly rooted in biological organizations, at various hierarchical spatiotemporal levels.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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