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Conference Report

Molecular Communication Approaches for Wetware Artificial Life: A Workshop Report

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Abstract: On 25 July 2023, a workshop entitled "Molecular Communication Approaches for Wetware Artificial Life" took place as a satellite event at the international conference ALIFE 2023 (The 2023 Conference on Artificial Life). In this report, we comment on the workshop by focusing on the main theme and the motivations that led us to develop this initiative. In particular, we highlight how recent progress in synthetic biology and in the study of molecular communication from an engineering perspective can be fruitfully joined to provide a powerful platform to develop frontier research lines in "wetware" Artificial Life. The talks presented at the workshop are briefly summarized. This report is, ultimately, an opportunity to promote an emerging field that calls for collaborative efforts of scholars from multiple disciplines, from chemistry to molecular biology, from communication engineering to nanotechnology, and up to those interested in more theoretical aspects about complex artificial systems that mimic natural ones.

Keywords: artificial life; synthetic biology; molecular communication; internet of bio-nano things; synthetic cells; artificial cells; nanomachines; unconventional communication; information and communication theory; chemical artificial intelligence

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1. ALIFE 2023

The 2023 Conference on Artificial Life (ALIFE2023) was held in Sapporo, Japan, from 24 to 28 July 2023 (http://2023.alife.org). The conference is the official annual event of the International Society for Artificial Life (ISAL), allowing researchers around the world to communicate their results and to look for networking and collaboration opportunities within the Artificial Life (ALife) community.

The theme of the conference, "Ghost in the Machine", refers to one of the most fascinating open questions in science, belonging to the ALife agenda since its inception in the late 1980s [1]. How to explain life, cognition, and consciousness—the greatest biology mysteries—by means of scientific theories and mechanisms? Contemporary researchers seek to unravel these age-old questions and explain what the "ghost in the machine" actually is, by making use of theoretical concepts such as emergence, agency, and autonomy and exploring the frameworks defined by the theories of complexity, self-organization, and information. The endless efforts made by scientists of all disciplines to answer these

fundamental questions are still only partially fruitful, although a significant amount of work has been carried out to answer them in the past decades. ALife, by the virtue of its peculiar inquiring methodology, the "synthetic" method (also known as the "constructive" method), seems to be one of the best arenas where such questions could definitively find the required answers. However, the task is rather challenging, and indeed, the clarification of the mechanisms giving rise to the "ghost in the machine", i.e., a "mind" (intended here in the broadest sense), is still elusive.

ALife investigations proceed by constructing models of the biological phenomenon under study, following the "explaining = constructing" paradigm. Often, this is epitomized by the famous Feynman quote "What I cannot create, I do not understand". ALife searches for the generative mechanisms that bring about life, cognition, and consciousness, and does so by looking for general mechanisms. It can be called the science of life "as it could be", not just of life "as it is". For these reasons, working in ALife also means exploring the entire range of possible systems that could give rise to lifelike phenomenology. Research in ALife, indeed, has been performed by exploring software and hardware models of living beings, and consequently has led to significant advancements in artificial intelligence (the software route) and in robotics (the hardware route). Only more recently, and thanks to the developments of synthetic biology and systems chemistry, the "wetware" approaches are consistently landing on ALife territory. Thus, the wetware route can also generate various models of biological phenomena and complement the other routes with respect to goals, approaches, and concepts. The common trait of these three approaches is their focus on the emergence of high-level phenomena from processes occurring at lower organizational levels.

Our interest in ALife specifically refers to the wetware route. As mentioned above, it is typically developed within synthetic biology and systems chemistry, and its roots go back to origin-of-life research. In particular, the central idea is the construction of artificial cells (ACs) intended as models of modern or ancient biological cells [2–15], or as biotechnological tools for novel applications [16–22]. The core idea of synthesizing an AC can be implemented by different approaches. For example, living cells are engineered by adding, subtracting, and modifying various molecular elements (e.g., genetic elements) so that the resulting bio-engineered cells display specific behaviors. The genetic circuitry and the metabolism of a cell can be "rewired" to control the cellular behavior. Such a route is often directed toward practical applications, but not necessarily. Indeed, in its most radical (and challenging) implementation, ACs are constructed by inserting a completely synthetic genome in cells deprived of their own natural genome. In this case, the achieved ACs are typically exploited as tools to decipher the minimal conditions of life in terms of the number of genes and functions [23–26]. According to another approach, cell-like systems are built from scratch, i.e., by assembling purified molecular components such as DNA plasmids, enzymes, energy-rich metabolites, lipids, amino acids, etc., to give rise to micro-compartments as those shown in Figure 1a. The assembly procedure can be accomplished thanks to technologies in the field of liposomes, emulsions, coacervates, and microfluidics [27–31], and also thanks to the recent developments of cell-free multi-enzymatic systems, for example, the ones capable of producing proteins from the corresponding genes [32,33]. Notably, the cell-like systems constructed by this second approach (often called "bottom-up") are, to date, not alive. Nevertheless, it should be remarked that contemporary research has made significant progress in the number and complexity of implemented functions. Consequently, the goal of constructing a very simple living AC from scratch, although extremely challenging, seems within experimental reach.

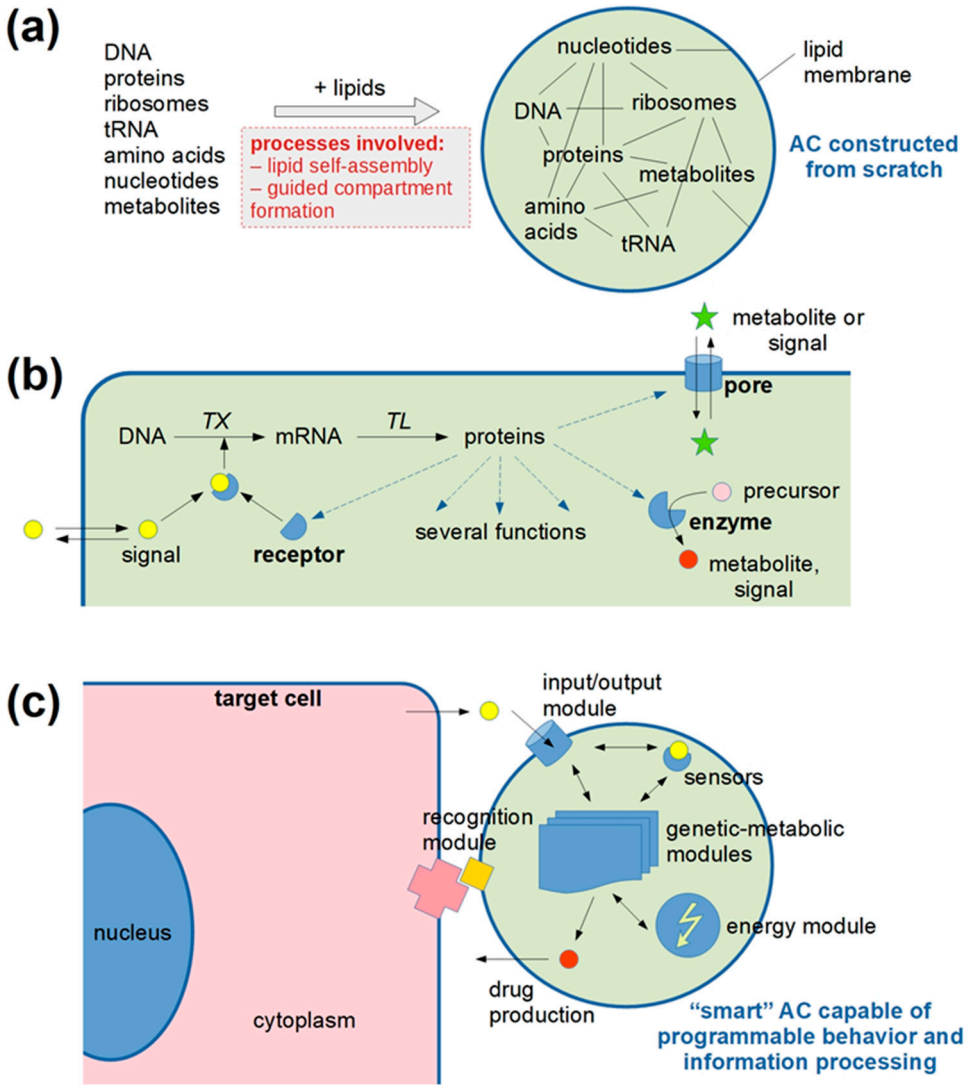

Figure 1. Artificial cells (ACs). (**a**) Artificial cells can be constructed by co-encapsulating molecules such as DNA, proteins, ribosomes, tRNA, amino acids, nucleotides, and metabolites in artificial micro-compartments often made of self-assembled lipids (liposomes). Lipids spontaneously form a membrane that enclose an aqueous volume containing the solutes of interest. Many methods can be employed to "guide" the formation of artificial cells. Once encapsulated, the compounds establish a dynamical chemical network by reacting with each other. The resulting systems, called ACs, resemble biological cells in structure and function, although with lower complexity. Notably, other micro-compartments, different than liposomes, can be used as well (e.g., coacervates, water-in-oil droplets, aqueous two-phase systems). (**b**) Some common biomimetic functions generated in current ACs. Proteins can be synthesized via transcription (TX) and translation (TL) mechanisms, thus involving RNA polymerase and ribosomes. Proteins with different functions such as membrane pores, catalytic enzymes, cytoskeleton ones, and receptors can be produced. The pores, for instance, allow low molecular weight solutes passing across the membrane; enzymes can synthesize metabolites or signals to be exported; receptors can bind signals that enter the AC; etc. (**c**) One possible use of ACs is based on their capacity of "perceiving" the environment and acting correspondingly. For instance, ACs can be conceived as smart drug delivery (or drug-producing) agents that travel in the body, recognize the target cells by the means of specific surface compounds, and have functional modules that allow the sensing of a stimulus produced by the target cell and produce and release a drug. ACs could generate chemical energy (ATP) from nutrients found in the blood stream. When fully programmable ACs will be available, additional functions can be imagined, such as delayed activation, response to combination of signals, self-destruction, etc.

A key AC function is the capability of communicating. ACs can exchange signaling molecules with other ACs and with natural biological cells because they can be endowed with encoding, producing, sending, receiving, and decoding devices. These devices are molecular, i.e., they are genetic elements that control AC activities, enzymes that synthesize signaling molecules, transmembrane pores that allow the passage of signaling molecules across the lipid membrane, and receptors that bind received molecules and translate the binding event in other biochemical processes (Figure 1b). In other words, communicating ACs are cell-like machines capable of manipulating information in the "chemical domain". Such a capability has attracted the attention not only of synthetic biologists but also—and independently—of the engineering community working since the early 2000s [34,35] on similar themes from the viewpoint of the information and communication theory (ICT). The resulting concept of molecular communication (MC) [36] is currently widely explored at the theoretical and experimental levels. Moreover, considering the possibility of interfacing artificial biological entities with each other, and with electronic devices too, the technological scenario of Internet of Bio-Nano Things (IoBNT) has been put forward [17,37,38]—highlighting its potential relevance for future nanomedicine applications.

Convinced that the convergence of AC synthetic biology and MC on the topic of communicating AC might represent a powerful multidisciplinary front for advancing wetware ALife (including, in the long term, the above-mentioned fundamental questions), we have organized a workshop entitled "Molecular Communication Approaches for Wetware Artificial Life", as a satellite ALIFE2023 event (the logo is shown in Figure 2). In particular, it was the first time that a workshop focusing on the intersection of synthetic biology and MC was presented to the ALife community. About thirty-five participants attended the workshop (in person and online) on 25 July 2023. The workshop call for papers is provided in Appendix A. Below, we report the synopsis of the four talks and some considerations that emerged from the discussion, intended as concluding remarks and future perspectives of these fields.

Figure 2. Logo of the ALIFE2023 satellite workshop "Molecular Communication Approaches for Wetware Artificial Life" (Sapporo, Japan, 25 July 2023).

2. Synopsis of the Talks Presented at the Workshop

In the Introduction, Pasquale Stano (University of Salento, Lecce, Italy) described the motivations behind the workshop, briefly recalling key theoretical principles of wetware models and the historical developments of the "communicating AC" theme. The unique features of (bio)chemical materials allow the construction of wetware ALife systems capable of displaying life-like behaviors such as growth, replication, control, evolution, and other complex bio-inspired patterns such as adaptation, plasticity, and autonomy. Wetware approaches can display deeper phenomenological and organizational relevance as models of biological systems, when compared to software or hardware approaches. To understand why, first of all, it should be noticed that the components of wetware systems

operate at the nanoscale, where different forms of energy have similar orders of magnitude and can be easily converted into each other [39,40]. In turn, this implies that wetware systems—like ACs—are affected by any physico-chemical factor, and their dynamics is unconstrained and genuinely self-determined. In other words, although ACs are generally designed in a precise manner (by a designer, as in the case of software and hardware), their existence is determined by internal processes. Only when these systems behave as (self-)organized wholes, they can persist in their environment. Moreover, they have a deep organizational relevance when their dynamics align with the autopoietic one [41–43], as discussed elsewhere [44–46]. Systems such as ACs and other similar ones (including those obtained via hybrid approaches) are the best candidate to engage communicative dynamics with biological cells. They speak the same language as the biological systems, operate by the same mechanisms, and are subjected to similar constraints. In order to reconstruct the birth of research on communicating ACs, some specific published studies have been recalled. In 2012, Stano et al. [47] presented a programmatic paper to sketch how chemical communication could be implemented in ACs that function by gene expression. The relevance of quorum sensing molecules and mechanisms was highlighted, as well as the possible dual interpretation of communication in AC systems according to the usual transmission-of-information metaphor (à la Shannon) or according to the "in-formation" perspective of perturbed out-of-equilibrium states of dynamical systems [48]. As sources of inspiration for the described program, the authors mentioned three main traces, whose relevance still stands, and are as follows: (1) the "nanofactory" scenario, presented in 2007 by Philip Le Duc, referred to smart drug delivery agents [49] (e.g., Figure 1c); (2) the MC perspective put forward by Tadashi Nakano and collaborators in the early 2000s [34–36]; and (3) the theoretical and experimental reports produced by UK researchers, in particular about a hypothetical Turing test applied to ACs by Leroy Cronin, Natalio Crasnogor, Ben Davis et al. in 2006 [50], and the first experimental model made of somewhat simple ACs that produce signaling molecules (AI2-like) successfully perceived by bacteria *Vibrio harveyi* [51]. In the years that followed, several pioneer investigations were carried out by employing gene-expressing ACs, quorum sensing signals, and sending and receiving mechanisms, including more recent sophisticated design [52–64]. One of the most referred study in the field is the bidirectional communication between ACs and bacteria, based on quorum sensing molecules, reported by Sheref Mansy and collaborators in 2017 [65].

Next, Jiewen Wang and Tadashi Nakano (Osaka Metropolitan University, Japan) presented a study entitled "An Agent-based Modelling Approach to Molecular Communication and Multicellular Structure Formation". Creating a large-scale structure from a group of bio-nanomachines is essential for engineering applications of molecular communication [66]. This work employs an agent-based modeling approach to study molecular communication and multicellular structure formation. It begins by introducing an agent-based model, where a bio-nanomachine is represented as an autonomous agent with state variables, incorporating typical cellular behaviors. It then uses this model to describe molecular communication systems that form specific multicellular structures. Finally, it showcases the design and implementation of a simulator for testing the agent-based models.

Yutetsu Kuruma (Japan Agency for Marine-Earth Science and Technology, Japan) provided a talk on the subject "Construction of Autopoietic Artificial Cell–Toward Construction of Self-Reproducing Man-Made Cells". Building artificial cells from molecules and genes is the biggest challenge not only in life science but also in science as a whole. Although many subcellular systems have been developed so far, such as gene expression, CO_2 fixation, energy production, etc., the phenomenon of self-reproduction has not been yet reproduced in wet approaches. Self-reproduction of cells is classified as self-growing and self-dividing. The self-growing of cells can be achieved by endogenously producing new phospholipids that form the cell membrane. Recently, Yutetsu Kuruma's group has constructed an artificial cell that synthesizes phospholipids inside phospholipid membrane vesicles [67]. The cells first convert small molecules, HCO_3^-, and acetic acids to fatty acids, then form phosphatidic acids in the cell membrane, resulting in the production of 10% of

the lipids forming the mother vesicle. The constructed artificial cell exhibits a structure very similar to the autopoietic system proposed by F. Varela and H. Maturana.

The final talk was delivered by Sasitharan Balasubramaniam (University of Nebraska–Lincoln, USA) on "Discovering Gene Regulatory Neural Network towards Realizing Biological AI". As the world is embracing artificial intelligence (AI), we are witnessing their integration into various devices and, in particular, low-powered devices. A question arises as to whether we can also integrate intelligence into artificial cells. The Gene Regulatory Network (GRN) of a cell specifies the relationships of gene expressions within a genetic circuit, which has a structure that also resembles a neural network. They have shown that a GRN can be converted into a Gene Regulatory Neural Network (GRNN), where their weights can be extracted from trans-omic data. This allows the pre-trained GRNN to be tailored to a specific environment as well as application, where further engineering can lead to improving computing reliability. In our work [68], we have shown how the GRNN computing can be extracted for *Pseudomonas aeruginosa* that utilizes chorismic acid as input and produces pyocyanin. We have also shown that through multi-cellular molecular communications, the population of cells can behave as a single perceptron.

A general discussion, focused on the possible synergy between the explored fields, closed the workshop. The aim was to identify how MC approaches could enrich research on communicating ACs and their use in ALife scenarios. Aspects related to fundamental issues as well as applied studies were mentioned. One of the most critical points that emerged from the discussion was the need of more occasions like seminars, workshops, and conferences where scholars coming from different disciplines could meet and discuss together, to favor collaborative pathways and the start of joint research programs.

3. Concluding Remarks and Perspectives

In reflection, the workshop "Molecular Communication Approaches for Wetware Artificial Life" set the stage for a new chapter in the convergence of synthetic biology, molecular communications, and ALife. The next steps will necessitate more rigorous collaborative efforts, bridging the gaps between disciplines, and a commitment to a shared vision. While challenges remain, there is a palpable sense of promise in the air [69,70].

The main challenge moving forward is to elucidate the latent connections between these fields with differing scales of abstraction and reductionism. While synthetic biology focuses on engineering the nitty-gritty of the biological world, molecular communication offers an elevated view, connecting functionalities and spatial organizations. The broader ALife perspective then hovers even higher, touching on vast abstractions like cognition. For a holistic understanding of "life as it could be", it is crucial to recognize the connections among these scales, and grasp how different ideas, tools, and questions continuously emerging from each research direction can have a functional role in this quest. We believe that molecular communications can serve as a key instrument to navigate a spectrum of autonomous agency or lifelikeness between "synthetic machines" and "life" and formulate new questions and explore potential answers right in the wetware domain where life has emerged.

Solutions to the above challenges will benefit from refining the efficiency and reliability of biological information transfer, addressing biosecurity and ethical concerns, standardizing wetware protocols, and ensuring biological compatibility for derived engineered life. The collaboration between disciplines offers promising applications in healthcare, environmental monitoring, biological computing, and beyond, provided researchers embrace multidisciplinary collaboration and the integration of intelligence and life.

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Conflicts of Interest: The authors declare no conflicts of interest.

Appendix A Molecular Communication Approaches for Wetware Artificial Life: Call for Papers

Recent advances in systems and synthetic biology constitute a basis for the achievement of the wetware approach for Artificial Life (ALife), in addition to hardware and software approaches. Developing ALife systems in wetware domains requires the use of chemical and biological materials to construct tools, devices, and systems capable of displaying life-like behaviors such as growth, division, adaptation, plasticity, evolution, autonomy, and other bio-inspired patterns.

While thermodynamic and kinetic laws governing (bio)chemical processes provide a basis to attack the complex task of devising systems that significantly contribute to ALife, it is also important to understand the organizational structure of ALife systems. It is often noted that the governing principles of organizational structures rely on a characterization of information flow. As a consequence, models and characterizations from information theory and communication theory will be useful in the study of organization in ALife.

The combinations of areas such as synthetic biology, systems chemistry, chemical reaction network theory, and chemical organization have already impacted ALife, as is often reported within the ALife community. On the other hand, the exploration of the so-called "bio-them-ICTs" (bio-chem-information and communication technologies) and the theories behind them, known as "Molecular Communications", has received—to date—limited attention in ALife.

The aim of this workshop is to fill this gap, providing an arena for discussing how current interest in chemical information and chemical communication can converge with ALife, especially in the context of synthetic biology and systems chemistry approaches. The field of molecular communications, recently developed from an engineering perspective, can provide valuable tools for achieving a higher degree of complexity in ALife systems, including the following: (i) synthetic/artificial cells or protocells and their assemblies and (ii) hybrid biological/artificial systems (e.g., synthetic cells that communicate with biological cells; hardware/software microsystems interfaced to biological systems; and networks made of both artificial and biological entities).

Organizers: Pasquale Stano (University of Salento, Italy), Michael Barros (University of Essex, UK), Malcolm Egan (Univ Lyon, INSA Lyon, INRIA, CITI, France), Murat Kuscu (Koç University, Turkey), Yutetsu Kuruma (Japan Agency for Marine-Earth Science and Technology, Japan), and Tadashi Nakano (Osaka Metropolitan University, Japan).

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