Toward artificial cells/living cells communication in hybrid ensembles

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Abstract

Recent progress in "artificial" (or "synthetic") cell research has allowed the construction of sophisticated systems capable of sending/receiving chemical signals to/from biological cells. This new scenario paves the way to conceptually new technologies and scientific investigations based on interfacing organisms (natural cells) and robots (artificial cells) and exploiting their built-in molecular communication capacity. The state-of-the-art is presented and commented from the perspective of Artificial Life and synthetic biology.

Interfacing wetware robots (artificial cells, ACs) with living organisms

Current research on artificial entities is classified as dealing with hardware, software, and wetware man-made constructs that model living organisms and are capable, at a certain extent, of behaving like living organisms.

The integration of hardware- and software-based approaches leads to modern robotics, whereas the wetware approaches lead to molecular systems endowed with high degree of spatial and functional cytomimetic organization known as "artificial" or "synthetic" cells.

Although artificial cells (ACs) were initially developed for assessing origins-of-life questions (i.e., ACs that allegedly resemble primitive cells), intensive investigation is currently carried out in the frontier field of synthetic biology, aiming at constructing systems that are "programmable", or that display life-like behavior with non-trivial complexity (e.g., collective properties, cognitive patterns, hierarchical organization).

ACs can be defined as those cell-like systems constituted by the encapsulation of (bio)chemical molecules inside micro-compartments. Their size can vary from 0.1 to about 50 μm and their constituents can be "primitive" compounds, modern biochemicals, or completely synthetic chemical molecules (Stano, 2019). The AC boundary is generally made of lipids, polymers, or other amphiphilic molecules. The current state of the art of AC technology, which consists in a combination of bio- and chemical microengineering, allows the construction of quite complex systems, which can be regarded, for all practical purposes, as *wetware robots*.

One of the most intriguing AC features is their capability of communicating with each other and with living cells by exchanging molecular signals. This very fundamental perception route lies at the origin of minimal cognitive patterns and it is common to all form of life. It is not surprising, then, that studies on molecular communications appeared in recent years (Lentini *et al*., 2016; Rampioni et al., 2019).

The relevance of these studies is multifold. On one hand, communicating ACs are *per se* a technological advancement in AC technology. Next, ACs capable of coordinating their operation by communicating with each other can display patters typical of biological communities (e.g., synchronization, collective behavior). Moreover, ACs capable of communicating with living cells can be used as wetware robots in nanomedical applications (e.g., next-generation smart drug delivery systems, Krinsky *et al*., 2018). Hybrid systems composed by living and non-living cells, or living cells and non-living organellae, can be envisaged, creating unprecedented "ecosystems" at the cellular level. Finally, because perceiving chemical signals is equivalent to a structural/functional coupling with the environment, such a research has also theoretical relevance in the epistemological discussion about the machine-likeness (heteronomy) versus the organism-likeness (autonomy) of ACs (Damiano and Stano, 2020).

In this contribution we would like to present the current state-of-the-art of chemical communication in AC research and discuss the several conceptual and applied implications related to those approaches.

Chemical communication

Bio-Chem-ICTs. The development of bio-chemical information and communication technologies (bio-chem ICTs) was developed by visionary scientists coming from the field of network engineering (Nakano *et al*., 2011, 2013). They were inspired by the built-in capabilities of natural organisms - as information processing systems - and devised a new scenario where chemical exchanges are the basis of information and communication technologies in the nanoscale and beyond. An entirely new communication theory and models (and information theory/models as well) stem from this radical shift of perspectives, from electric to chemical signals, essentially due to two facts: the "granular" and stochastic features of molecular signals, and the intermingled functions attributed to molecular parts – which are often at the same time encoders, signals, and decoders (ultimately originated by the embodied nature of molecular systems).

ACs potential and state of the art. The innovative territory of bio-chem ICT research offers opportunities for exploiting the embodied information processing capability of ACs, developed according to bottom-up synthetic biology (Stano *et al.*, 2012). In particular, the construction of ACs based on gene expression inside liposomes is the appropriate technology for pioneering works.

The list of exciting published studies highlights the current progress in the field (Rampioni *et al*., 2019). For example, ACs acting as "translators" for the bacterium *Escherichia Coli* language have been reported (Lentini et al., 2014). Adamala *et al*. (2017) built ACs containing engineered genetic circuits that can be triggered from chemicals capable of permeating the ACs. As a result, the ACs synthesize pore-forming proteins that allow the emission of a signal molecule. In a landmark paper, Sheref Mansy and collaborators reported for the first time a bi-directional communication between ACs and natural cells, exploiting quorum sensing mechanisms in *Vibrio fischeri*. It was shown a sort of unprecedented mini-dialogue between the biological and the artificial entities, based on gene expression (Lentini *et al*., 2017). Even more recently, the same group reported how properly designed ACs respond to the presence of a small molecule in the environment by synthesizing and releasing a protein signal, the brain-derived neurotrophic factor. The latter is capable of affecting engineered human embryonic kidney cells and murine neural stem cells, eliciting phenotypic changes, e.g., neuronal differentiation. (Toparlak *et al*., 2020)

It is then evident the ACs potential if the possibilities in terms of design, modeling, control, programmability, and modularity is considered.

Further exploiting bacterial quorum sensing mechanism. We have been actively involved in experimental research on ACs that communicate with biological cells (Rampioni *et al.*, 2018), focusing on the *Pseudomonas aeruginosa* bacterium (a species that causes hard-to-eradicate infections, and it is a major target for innovative therapies). Our initial goal was the demonstration of ACs/bacteria interaction via chemical communication, a first step of our long-term project. Figure 1 represents our first AC-bacterium communication system. We demonstrated the occurred AC-to-living cell communication by means of a bacterial *quorum sensing* signal, called C4-HSL (a well known molecule used by bacteria to coordinate their behavior). In particular every step of the mechanism depicted in Figure 1 was rigorously demonstrated. ACs, whose typical size was 3.5 μm, were able to produce the enzyme RhlI within one hour and induce a specific pattern of gene expression in the receiver bacterium in similar time scale. In addition to luminescence (used as reporter), five relevant signal-related genes were activated, showing that bacteria perceive the signal produced by the ACs as if bacteria peers produced it. With surprise, we also verified that the co-incubation of ACs and bacteria was possible only in gel media, because bacteria activated also a chemotactic response culminating – in liquid media – in the attack and digestion of ACs. By comparing this observation with other studies where other bacteria were used (Lentini *et al*., 2017) we ascribe this peculiar pattern to the specific nature of *P. aeruginosa*.

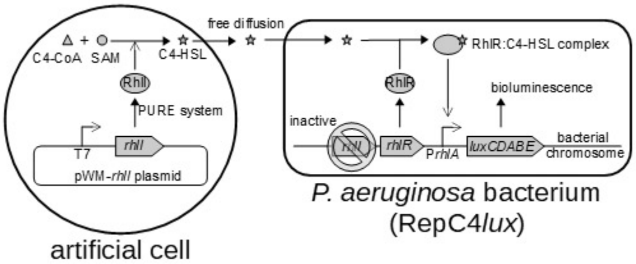

Figure 1. Design of an AC sending a quorum sensing signal molecule to the bacterium *Pseudomonas aeruginosa*. The sending system is based on the production of the signal molecule C4-HSL by the enzyme RhlI, which is in turn produced by a transcription-translation mechanism inside the AC, operated by the PURE system. The enzyme convert two precursors (C4-CoA and SAM) into the actual signal C4-HSL, which spreads through lipid membranes, diffuses into the medium and reaches *P. aeruginosa* cells. The receiving cells – called RepC4*lux* – contain a genetic reporter device for C4-HSL-induced bioluminescence and a mutation inactivating the *rhlI* gene, so that they cannot produce C4-HSL. C4-HSL produced by ACs binds to the receptor RhlR, so that the complex RhlR:C4-HSL triggers *luxCDABE* transcription and bioluminescence emission. Taken from (Rampioni *et al*., 2018).

Outlooks

Interfacing natural cells with ACs is one of the scenarios were organisms and robots interact in a bio-hybrid perspective. Here, wetware artificial systems and living cell exchange chemical information, communicating to each other. Progress in AC technology will lead to programmable and minimally cognitive systems that can impact on future nanomedical applications. For example, it has been already shown that ACs can produce an antimicrobial peptide (Bac2A) in response to quorum sensing signal molecules sent by bacteria (the result is bacteria death, Ding et al., 2018). In a more sophisticated nanomedicine scenario (but deprived of molecular communication), ACs were injected inside a mouse body, producing a toxin in order to kill cancer cells (Krinsky et al., 2018). The final goal, in this respect, points to the construction of intelligent drug delivery systems, as evidenced some time ago by Leduc *et al.* (2007). In that perspective paper, the Authors put forward the concept of "nanofactory", i.e., a sort of AC that travels into the body, reaches its target tissue (recognized by its surface antibodies), and produces *in situ* the therapeutical chemical of interest only where/when needed. This control is achieved by sensory elements that activate intra-AC synthetic modules. Finally, a self-disruption mechanism was also proposed.

In addition to these relevant applications, communicating ACs can play an important role as a tool for assessing long-standing theoretical questions, such as the issue of being able to construct minimal cognitive systems, and attempting to build new forms of AI based on chemical systems.

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