En route for implanting a minimal chemical perceptron into artificial cells

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Abstract

This paper describes a potentially rewarding research program aimed at designing, modeling, analyzing and experimentally realizing artificial cells in the wetware domain endowed with a 'neural network'-like module for achieving minimal perception. In particular, we present a possible implementation based on bacterial phosphorylation signaling networks (dubbed as "phospho-neural network" by Hellingwerf and collaborators in 1995). At this initial stage only preliminary discussions are possible. The scenario we devise minimizes unrealistic assumptions and it is based on the state-of-the-art of contemporary artificial cell technology. This contribution is intended as a plan to forster the construction and the theoretical analysis of next-generation artificial cells.

Wetware Artificial Cells

Tremendous advancements in artificial cell (AC) research have been reported in the past years, thanks to the joint efforts of several communities (for example, the MaxSynBio, Build-a-Cell, BaSyC, FabriCell initiatives), attracted by the novel idea of constructing cell-like systems by assemblage processes. The latter differ from conventional synthetic biology because a "bottom-up" approach is employed. Complex cell-like structures are literally built from scratch by employing molecules such as DNA, RNA, ribosomes, enzymes, lipids, etc., or allegedly primitive molecules (fatty acids, ribozymes, short peptides, …), or even completely artificial molecules (amphiphilic molecules and/or polymers, *ad hoc* designed transition metal-based catalysts, organocatalysts, etc.). A distinctive element that characterizes bottom-up approaches to ACs is the focus on basic scientific questions (e.g., what is the minimal complexity for life? What is the minimal genetic information required to sustain cellular self-reproduction? Can we build a minimal cognitive chemical system?). Because AC technology does not resemble any other existing technology, the entire field is experiencing a momentum and a wide variety of results are continuously reported. It is possible to bet about the central role that AC technology could play in future, if properly developed in theoretical and applied science arenas.

Following the pioneering phase of the early 2000s, recent efforts have shown that it is possible to design and construct ACs that perform a variety of cell-like functions. It is beyond the scope of this abstract to summarize the state-of-the-art (interested readers can find more information in excellent recently published reviews [1-4]). For the present discussion it is enough to say that today it is possible to reconstruct several basic cellular functions, including those related to crucial mechanisms such as gene expression, DNA replication, signaling, transport, bioenergy generation, morphological transformations, small enzymatic pathways, transmembrane protein functions. Until now, most of these processes have been demonstrated one at a time, while one of the next challenging goals refers to the integration of these different "modules" into a whole, in order to reach higher complexity levels.

In this contribution, we will make one step forward, and describe a new research goal in AC research. We base our discussion on realistic expectations about what it will be possible to build in the near future, under the hypothesis that conditions will be found to allow the different coexisting "modules" of this hypothetical system work smoothly together.

A Wetware Embodied AI?

A fruitful scientific field for developing the present research plan is "Embodied AI": the area of AI that focuses on the role played by the body in cognitive processes, and thus uses, as synthetic models of natural cognitive systems, "complete agents" – i.e., agents that, differently from computers, have a body, and, through this body, can perceive and react to their environments, accomplishing cognitive tasks [5,6]. Since its birth in the late 80s, Embodied AI has been working on modeling cognitive embodied agents mainly through hardware models – electromechanical robots. Intrigued by the opportunity of developing Embodied AI in the *wetware*

domain, we therefore asked whether, and to what extent, ACs can constitute a platform for the theoretical and experimental investigation of a "Chemical Embodied AI".

In this respect, there are different interesting modeling options. The first one derives from the fact that ACs are expected to be developed within systems-level frameworks, e.g., autopoiesis [7]. Accordingly, if ACs were autonomous, they would display a cognitive dimension [8,9], and then would represent very interesting models for Embodied AI. The problem with this approach is that current ACs do not yet realize the *organizational closure*, the prerequisite for autonomy [10]. A second compelling option can be conceived in terms of ACs that do not have an organizational closure in itself, but their modular organization is thought as a part of a larger organizational closure (whose construction must be approached stepwisely).

Here we consider the implantation of an upstream AI "module" to control gene expression inside ACs. In particular, Neural Networks (NNs) draw our attention. In AI, NNs are generally implemented in the logical domain of software [11]. Our main research question becomes: is it possible to devise chemical NNs (*chemical perceptrons*) implemented in the wetware domain and in particular in the field of Embodied AI? And, by means of modeling, what is the minimal NN complexity that would generate interesting behavior?

Phospho-Neural Networks

A brief review on the attempts of constructing chemical NNs can be found in [12,13]. Here we will move straight to the point that we consider of relevance for AC technology. Hellingwerf and collaborators, in 1995, have put forward a lucid discussion about interpreting the bacterial signaling systems (the so-called two-component systems, TCSs) as chemical NNs [14]. These authors actually called them "phospho-NNs", because their functioning is based on molecular phosphorylation.

TCSs enable bacteria to sense, respond, and adapt to their environments, letting the cell perceive chemical signals present in their surroundings. In a typical TCS, a membrane protein (sensor, S) with histidine kinase activity catalyzes its autophosphorylation in the presence of a stimulus. Next, the sensor is capable of transferring the phosphoryl group to a response regulator (R), which can then affect cellular physiology by regulating gene expression or by modulating protein activity (Figure 1a). This series of reactions can be interpreted as a transmission of information, from outside to the genome (and, in turn, to the profile of proteins present in the cell).

Generally each TCS transmits information in a linear way, i.e., the *i*-th signal is sensed by the *i*-th cognate sensor, which self-phosphorylates and in turn phosphorilates, later, the *i*-th cognate regulator, affecting one or more gene expression mechanism(s). To function properly, however, some TCSs involve convergent or divergent branched pathways. Moreover, and this is a relevant observation for our proposal, *crosstalk* between TCSs is also possible, at the level of sensors and/or regulators, leading to a NN-like system (Figure 1b). Normally, discussions about sensing and the molecular biology of well-functioning TCSs emphasize in which conditions crosstalk is reduced or eliminated. In the new perspective of implanting artificial TCSs inside ACs (Figure 1c), however, crosstalk becomes essential, and thus the interest goes to conditions for favoring and *controlling* its occurrence [15].

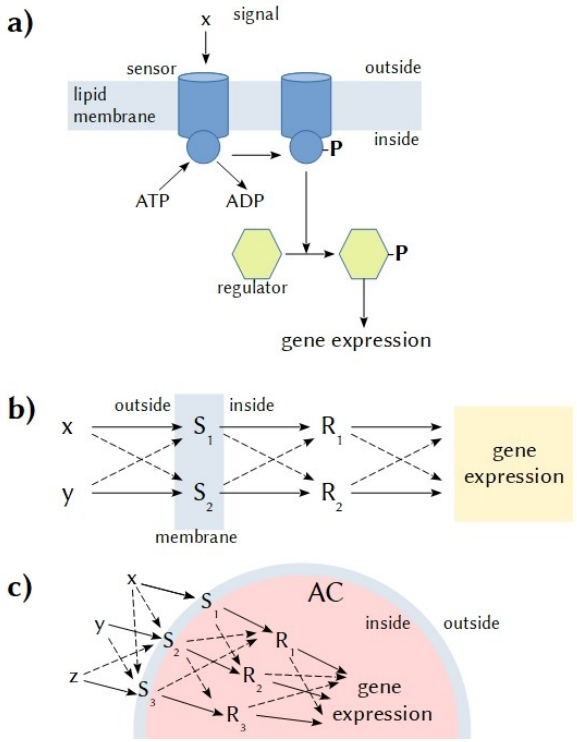

Figure 1. Phospho-NNs inside ACs. (a) The structure of a TCS; P represents the phosphate group. (b) When two TCSs crosstalk (shown dashed arrows), a NN-like system is generated. (c) Schematic drawing of a phospho-NN, based on three TCSs, implanted into an AC.

At this initial stage we identify two relevant goals, summarized as it follows. (1) Analysing the feasibility of intra-AC phospho-NNs, which will depend both on the right selection of TCSs and on the technical capability of building ACs endowed with the required molecular components. Critical elements are the transmembrane histidine kinase sensors, which should be reconstructed in functional form and in the desired orientation. Recent results on transmembrane protein reconstitution in ACs [16-18] constitute the starting point for design and experimentation. (2) With respect to numerical models, recognizing that chemical embodiment implies an intrinsic heterogeneity of AC physicochemical microenvironment. In turn, this generates the coexistence of many conformations (and activities) for the macromolecules constituting the phospho-NNs. The suggestion is that chemical perceptrons should be modeled by placing side-by-side both binary and not binary (fuzzy [19]) input/outputs.

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