

Molecular Communication Technology: General Considerations on the Use of Synthetic Cells and Some Hints from In Silico Modelling

Fabio Mavelli¹, Giordano Rampioni², Luisa Damiano³, Marco Messina²,
Livia Leoni², and Pasquale Stano²(✉)

¹ Chemistry Department, University of Bari, Bari, Italy

² Science Department, Roma Tre University, Rome, Italy
pasquale.stano@uniroma3.it

³ Centre for Research on Complex Systems, University of Bergamo, Bergamo, Italy

Abstract. Recent advancements in synthetic biology pave the way to the design and construction of synthetic cells of increasing complexity, capable of performing specific functions in programmable manner. One of the most exciting goal is the development of a molecular communication technology based on the exchange of chemical signals between synthetic and natural cells. We are currently involved in such a research program. Following our previous contributions to WIVACE workshops (2012–2013), here we present the project, and discuss some general considerations on the use of synthetic cells for developing novel bio-chemical Information and Communication Technologies (bio-chem-ICTs). Moreover, by analysing in detail a mathematical model of synthetic cell/natural cell communication process, we provide some hints that can be valuable for the next experimental steps.

Keywords: Bio-chem-ICTs · Molecular communication · Quorum sensing · Synthetic biology · Synthetic cells

1 Introduction

Synthetic biology (SB) can be defined as (a) the design and construction of new biological parts, devices, and systems, and (b) the re-design of existing, natural biological systems for useful purposes (<http://syntheticbiology.org>). Classical SB approaches, therefore, generally consist in the integration of biology and engineering [1, 2], and aim at designing biological organisms (generally micro-organisms) by the addition, elimination, modification, and redesign of parts and circuits. However, a novel SB paradigm is emerging, and it foresees in the construction of synthetic cells from their components (Fig. 1a). Such an approach is often referred as “bottom-up”, and consists in assembling synthetic cells (from molecular components like DNA, proteins, lipids, etc.) endowed with a minimal degree of complexity, yet capable of displaying cell-like functions - and ultimately of being alive [3].

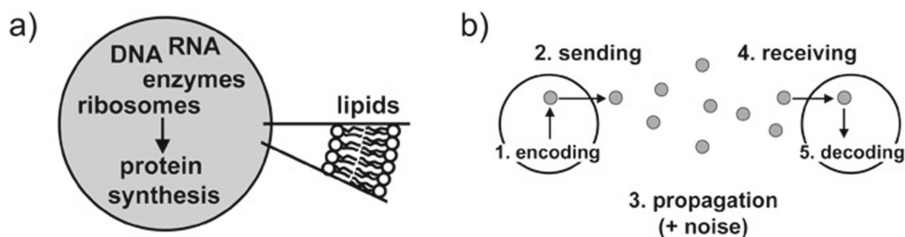


Fig. 1. (a) Synthetic cells [3] can be built by encapsulating the minimal number of biomolecules inside lipid vesicles (liposomes). To date, synthetic cells can synthesize functional proteins with good efficiency, but are unable to generate energy, grow/duplicate. (b) Outline of a molecular communication process based on the diffusion of signal molecules from a sender cell and a receiver cell, sharing the general scheme of a Shannon communication model [7]. Reproduced from [6] with the permission of Springer.

The construction of synthetic cells is rapidly progressing owing to the recent convergence of liposome technology and cell-free transcription-translation (TX-TL) systems. In particular, synthetic cells are obtained by encapsulating TX-TL systems inside lipid vesicles (liposomes), together with the gene(s) of interest. The encapsulated TX-TL system produces the proteins of interest, which in turn perform the desired function within the synthetic cells. When built in this way, synthetic cells become a very interesting tool for investigating scientific open questions (for example, the origin of life) and for developing novel biotechnological tools. Their design is versatile and modular, they are bio-compatible, and, importantly, fully programmable (at least in principle). By constructing well-designed synthetic cells it becomes possible to observe, study and control the dynamics of biochemical, sensorial or regulatory networks without the interference of background processes that are always present in biological cells.

Molecular communication (Fig. 1b), being a fundamental and widespread process in biological systems, is a fascinating target function for synthetic cells [4–6], that has not been realized yet. By processing molecular signals, synthetic cells could effectively communicate with each other and, intriguingly, with biological cells. This would pave the way to the construction of novel microscopic systems based on embodied self-production and self-maintenance (autopoiesis), and operating in the domain of bio-chemical Information and Communication Technologies (bio-chem-ICTs) [7–9]. Such a project, in our opinion, represents a starting point not only for future advancements in nanomedicine [10], but also for theoretical and experimental developments concerning the concept of minimal (embodied) autopoietic cognition, which would open novel perspectives in the synthetic study of natural cognition and in artificial intelligence [11]. Synthetic cells can be considered as molecular robots that can be functionalized with sensors and actuators. In contrast with electromechanical robots, however, synthetic cells functions are embodied in the structure of their molecular components, which continuously and parallelly interact with each other on the basis of diffusion, molecular recognition, and cooperative behaviour – without the need of a central directional unit.

In previous contributions we introduced the central idea of endowing synthetic cells with minimal communication devices, and proposed a blueprint for the experimental construction of such systems. Following the usual synthetic biology work-flow (design, modelling, construction), we have also recently presented an *in silico* model of unidirectional synthetic-to-natural cell communication [6]. Here we would like to further promote our vision, presenting the synthetic communication project. Firstly, we will shortly introduce a synthetic biology approach for developing synthetic cells, and review it according to the general concepts in molecular communication technologies, which have been recently illustrated by T. Nakano, A. W. Eckford, and T. Haraguchi in their monography [9]. Then, we will present a mathematical model that describes synthetic cell/natural cell communication [6], supplied with a detailed analysis of its parameters.

2 The Experimental Approach

In the past two years we have been working on the extension of the current synthetic cell approaches toward the design of molecular communication systems based on the synthesis and the exchange of diffusible chemicals. The first experimental approach is due to Ben Davis and collaborators [12], who reported a simple chemical communication system based on the encapsulation of the so-called formose reaction inside liposomes. As a result of internal reaction, a chemical was produced by the synthetic cell. Once released in the medium, this chemical compound can reach bacteria and activate a biological response (chemoluminescence). Davis' synthetic cells, however, lack modularity, programmability and variability in their design, because they are based specifically on a certain particular chemical reaction (the formose reaction). A synthetic biology approach appears much more powerful. In fact, since it is based on protein synthesis inside liposomes [13], almost all types of synthetic cells can be envisaged, and their construction made possible by employing biological elements like enzymes, receptors, transcription factors, etc. These molecular components are endowed with a natural chemical processing capability and can be combined modularly so to construct multifunctional devices.

2.1 Synthetic Cells

Synthetic cells (or, more precisely, semi-synthetic minimal cells [3]), are cell-like compartments based on biochemical machineries encapsulated inside lipid vesicles (liposomes) (Fig. 1a) [14]. In order to build synthetic cells capable of non-trivial behaviour, several macromolecules (DNA, RNA, proteins) must be inserted inside liposomes. This can be conveniently done by employing a rather novel preparation method called *droplet transfer* method [15–18], which allows an efficient encapsulation of solutes inside giant vesicles (GVs). In turn, GV's are conveniently manipulated and visualized thanks to their very large size (diameter $>1\ \mu\text{m}$). As TX-TL machinery, the PURE system [19] is often used because

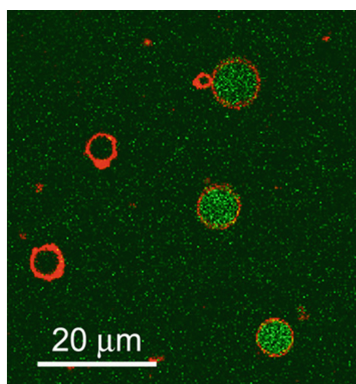


Fig. 2. PURE-system containing synthetic cells based on giant lipid vesicles are able to produce the green fluorescent protein in their inner aqueous core (green fluorescence). The liposome membrane (red fluorescence) has been stained with Trypan Blue. Reproduced from [6] with the permission of Springer (Color figure online).

it contains the minimal number of molecular components necessary for *in vitro* protein synthesis, all well characterized and present in known concentrations. This feature makes the PURE system the perfect tool for synthetic biology, *i.e.*, according to the concept of *standard parts* (<http://partsregistry.org>). Several proteins and enzymes have been synthesized inside lipid vesicles of various type by using the PURE system [14], including membrane enzymes [20], see Fig. 2. Thus, current synthetic cell technology allows the construction of cell-like compartment of non-trivial complexity that can perform specific functions. It is realistic to think that the enzymes, the receptors and other molecules that take part to encoding/sending or receiving/decoding circuitry can be synthesized inside synthetic cells by TX-TL reactions.

This scenario corresponds to the construction of synthetic cells capable of producing and manipulating chemical information, and therefore establishing chemical communication between each other or with natural biological cells. Since the machineries for performing these actions (computations) are themselves produced in – and by – the synthetic cell, possibly under control of other chemical information systems, it is evident that synthetic cells, when properly developed, will represent the prototype of autonomous artificial systems. Such an approach is therefore one of the best candidates for a novel paradigm in artificial life and artificial intelligence.

2.2 Bacterial AHLs as Molecular Communication System

In the context of developing communicating synthetic cells with minimal complexity, we believe that exploiting the bacterial communication systems is the most advantageous choice to start with. The bacterial world offers a plethora of communication systems well characterized at the molecular level,

hence exploitable for synthetic biology approaches. Indeed, most bacterial genera coordinate their activities at the population level via a widespread inter-cellular communication system known as quorum sensing (QS) [21–24]. Briefly, QS relies on the synthesis, secretion/diffusion, reception and decodification of signal molecules by members of a bacterial community. In order to design a minimal communication mechanism inspired from cell communication protocols, a proper design in terms of choice of molecular parts and devices to be implemented in synthetic cells is needed. QS systems based on *N*-acyl-homoserine lactones (AHLs) as signal molecules are promising candidates to establish a synthetic/natural communication system. In particular, AHLs synthesis requires a single step reaction usually driven by a single enzyme, and, when they permeate into the cell, AHLs are perceived by a cytoplasmic receptor. Moreover, AHLs are considered to be free-diffusible across biological membranes.

3 Molecular Communication Technology

The technology of molecular communication is a novel research branch in rapid expansion. Essentially, it deals with the manipulation of chemical information in biology and chemistry, and promises interesting application, especially in nanomedicine. Among many others, Tadashi Nakano and collaborators presented a series of programmatic papers [25] on the employment of molecular communication mechanisms that intriguingly inspired us. In particular, we were surprised to see how well our synthetic cell approach [3, 14] fits with the scenario presented by other authors.

Molecular communications occur in all biological systems at the intra-cellular and inter-cellular levels. There are several examples taken from unicellular and multicellular organisms, for example the regulation of transcription, the functioning of surface receptors, the pathways of calcium signalling, the hormonal and neuronal signalling, and several others. In the context of bio-chem-ICTs, molecular communication is implemented in nano- and micro-machines (systems) – made of biological or chemical materials – that can perform computation, sensing, actuation. Shannon theory also applies to molecular communication, which requires encoding, sending, propagation, receiving and decoding steps. However, in contrary to familiar telecommunication systems, molecular communication is endowed of peculiar traits that are summarized in Table 1. It is useful to quickly comment, following the discussion presented in [9], some of the entries in Table 1, with specific reference to the project proposed here.

The first three entries are self-explanatory. The creation of a communication channel between synthetic and natural cells is necessarily based on molecules, those that can be recognized by the biological cell as meaning-carrying ones. The synthetic cell, as a whole, act as a complex micro-machine, composed by several thousands of biomolecules belonging to more than 100 different species. For example, synthetic cells like those shown in Fig. 2, with diameter around 5 μm , contain several thousands of ribosomes and TX-TL enzymes, millions of low molecular weight species. The membrane – which is itself built by million

Table 1. Comparison between traditional (electro-optical) and chemical communications, adapted from [9,25]

| | | Traditional communication | Molecular communication |
|----|---------------------------|---------------------------|--------------------------------------|
| 1 | Communication carrier | Electromagnetic waves | Molecules |
| 2 | Devices | Electronic devices | Bio-nanomachines |
| 3 | Signal type | Electronic and optical | Chemical |
| 4 | Encoded information | Voice, text, video | Phenomena and chemical states |
| 5 | Behaviour of the receiver | Interpretation | Chemical reactions |
| 6 | Propagation media | Air/cables | Aqueous |
| 7 | Propagation speed | Speed of light | Extremely slow |
| 8 | Propagation range | m-km | nm- μ m |
| 9 | Energy consumed | Electrical (high) | Biochemical (low) |
| 10 | Other features | Accurate | Bio-compatible, parallel, stochastic |

lipid molecules kept together by non-covalent interactions – encloses all these molecule together and confines them in the inner vesicle lumen. This complex assembly operates within the realm of physics and chemistry but its functions are biochemically controlled, *i.e.*, information is processed as it happens in biological cells. It can be designed so to enzymatically produce an output (a molecule) that convey a certain meaning for the biological partner. This is a chemical signal (entry nr. 3) embodied as a molecule with well-specific three-dimensional structure.

Once synthesized and released in the environment, the chemical species will diffuse around creating a three-dimensional “chemical field” which can excite the sensors of the receiving biomachine, for example a living cell. The signal molecule will communicate, to the receiver, the chemical state of the sender (*i.e.*, a state where the production of a certain *signal synthase* is ON). In more complex examples, depending on the cellular internal state, different kinds of signals, combination of signals, or signals of different amplitudes could convey the message, see Fig. 3.

Moving to entry nr. 5, the chemical signal will be able to trigger one or more chemical reactions in the receiver cell, and these reactions will constitute the response to the signal.

Low speed and short range are two typical features of molecular communications in aqueous media (entries 6–8). In fact, when the communication is based on a free diffusion mechanism, as in the case of synthetic cell sending a signal molecule to biological cell, it is expected that the distance between the sender and the receiver would play a major role. The average time τ required for a molecule to propagate over a distance x can be described by the equation $\tau \approx x^2/D$, where D is the diffusion coefficient. Therefore τ scales with the square of the distance, and doubling the distance means quadruplicating the travelling time. In turn, D depends on the size and shape of the molecule. Large molecules, having low D , will travel slower than small ones. Since in our case the signal molecule will be a quite small one (molecular weight of about 200–400 Da), using

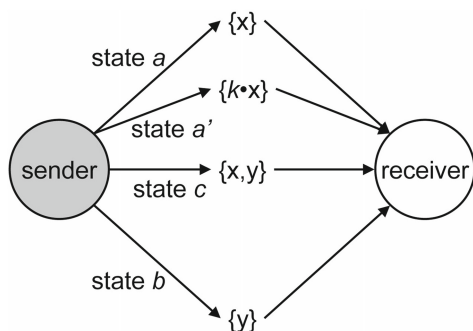


Fig. 3. Illustration of four simple ways of meaning-generation in molecular communications between synthetic/synthetic or synthetic/natural cells. State a is encoded by the signal molecule x ; state a' by a different amount of signal molecule $k \cdot x$; state b by the signal molecule y ; state c by a combination of x and y . Note, however, that it could be difficult to distinguish between state a and a' because the amount of signal molecule x in a certain point of space depends on the distance of the sender(s) from that point. See also [9] for additional examples (*i.e.*, signalling with timing).

an estimated diffusion coefficient D of $500 \mu\text{m}^2/\text{s}$, it turns out that one molecule would take about $\tau \sim 20$ s when $x \sim 100 \mu\text{m}$ (an estimate of the expected distance between senders and receivers in a plausible experimental setup, see below). The accumulation of signal molecules in the receiver, which is necessary to trigger its response, will depend, therefore, by the geometry of the system, and also by the rate of signal production. Considering that biological responses of the TX-TL type have characteristic response times of the order of 10^0 – 10^2 min it can be concluded that free diffusion in aqueous medium, despite its slowness, will probably not limit the mechanism we would like to exploit (Fig. 1b). As it will be shown below, however, the steps before and after the free diffusion in the medium can be rate-limiting. Signal molecules must be released by the transmitter and taken up by the receiver, and in both cases they have to cross the boundaries (the lipid membranes) of these systems.

From the energetic viewpoint (entry nr. 9), as all biological micro- and nanomachines, synthetic cells are powered by chemicals. In our specific case, TX-TL reactions consume nucleoside triphosphates to build the messenger RNA as well as to carry out protein synthesis. The energy requirement for TX-TL reaction, therefore, is significant. Since synthetic cells do not autonomously produce chemical energy, energy-rich compounds must be included in the encapsulated reaction mixture, and this is the usual way to proceed in current research. Alternatively, energy-rich compounds could be given externally by providing a route for entering the synthetic cell. According to previous results [26] it is sufficient to assemble an α -haemolysin pore on the vesicle membrane – Fig. 4 – to allow the entrance of low molecular weight compounds (<3 kDa), such as the energy-rich compounds, without releasing macromolecules. Another possible, more complex but also more intriguing solution could be endowing synthetic cells with

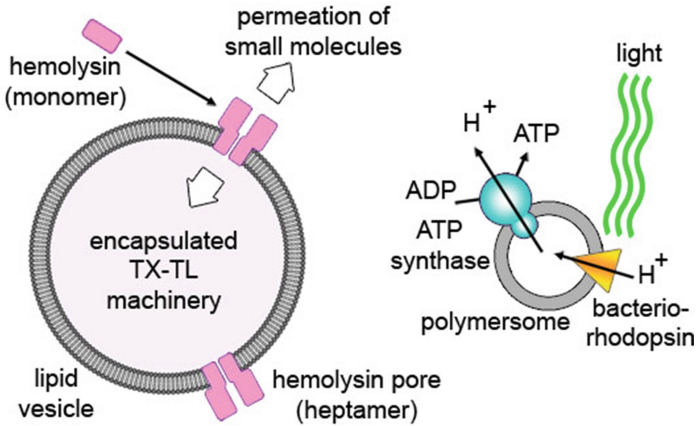


Fig. 4. (a) Formation of α -hemolysin pore allows the exchange of small molecule across the liposome membrane, as shown in [26]. (b) The encapsulation of energy-producing polymersomes [27] inside a giant vesicle could provide to the latter a sort of organelle that produce energy (ATP) after irradiation.

an energy producing system. Nano-polymersomes functionalized with bacteriorhodopsin and F_0F_1 -ATP synthase have been described [27]. These particles, when inserted inside GV containing the TX-TL mixture, could synthesize ATP after formation of a local chemiosmotic H^+ gradient, exploiting light energy as primary source (Fig. 4). In other words, such particles should function as synthetic energy-producing organelles inside the synthetic cell. Note that the assembly of multi-compartment systems with this architecture (vesosomes) is already known [28,29].

The final three features concern stochasticity, parallelism and biocompatibility (entry nr. 10). The latter property is well evident and do not require any comment (being synthetic cells composed of biomolecules).

Parallelism is obtained by letting different molecular circuitries working simultaneously, and this concept is linked to the synthetic biology issue of modularity. Synthetic cells can be designed and built in order to have modular subsystems capable of performing operations with minimal interference. The first example of modular process is given by the TX-TL reactions, which in the PURE system are implemented in four modules, namely, 1. transcription, 2. translation, 3. aminoacylation, 4. energy regeneration. Genetic circuits can be designed in order to operate in parallel [30], and their performance can be fine tuned, at least in principle, by accurate re-design of the promoter regions.

Stochasticity is an inherent characteristic of molecular nano- and micro-systems [31]. In the context of synthetic/natural cell communication, stochastic effects play a role at three different levels. Firstly, stochastic effects will determine the composition of synthetic cells during their assembly (extrinsic stochastic effects). It is well known that the construction of synthetic cells rarely

brings about very homogeneous populations. The compositional diversity among individual vesicles, *i.e.*, the diversity of their inner content [32], is high and generates – in turn – heterogeneity in the biochemical mechanisms of encoding, production and exportation of chemical signals, as well as signal reception and processing. In principle, this source of stochasticity can be reduced by assembling synthetic cells with methods allowing a more accurate control of synthetic cell content. The intrinsic stochasticity of molecular reaction networks is the second aspect, and applies both to synthetic and biological counterparts. This cannot be eliminated because derives from the unpredictability of molecular motion and of the reaction events, especially when systems are built with a limited number of molecules. Intrinsic stochasticity generates different reaction dynamics even within a set of identical molecular machines. Thirdly, stochastic factors affect the efficiency of molecular communication channel and of the signal-to-noise ratio. Free diffusion is a stochastic phenomenon and the time required to the signal molecule to reach the target is a random variable. The signal-to-noise ratio is also affected by stochasticity, and a robust molecular communication system is obtained by sending a high number of signal molecules (signal strengthening). The noise, on the other hand, is associated to the environmental presence of molecules with a structure similar to the signal molecule, or of signal molecules present in the environment following a previous communication event. The degradation or the removal of these unwanted molecules can therefore improve the efficiency of molecular communication (noise weakening).

4 In Silico Modelling

The use of mathematical models is a major feature of synthetic biology, because a mathematical description of the system under study helps to quantitative thinking and supports a bioengineering approach. The system under study consists in a synthetic cell sending a chemical signal to a bacterium (Fig. 5).

The model refers to a realistic case of synthetic cells producing a short chain and therefore freely permeable AHL that is recognized by bacteria as a chemical messenger. In particular, after PURE system encapsulation, vesicles should be able to produce the RhlI enzyme, which in turn will catalyse the synthesis of *N*-butyryl homoserine lactone (C_4 -HSL) from its precursors. The biological partner is instead an engineered *Pseudomonas aeruginosa* strain. The system is composed by a number of bacteria N_{bact} and of synthetic cells N_{sc} (in a 320-to-1 fixed ratio) dispersed in a volume of 0.2 nL. Synthetic cells have a diameter of about 5.4 μm . The processes occurring inside synthetic cells and inside bacteria are described by a set of ODEs (Eqs. 1–10), shaped according to Michaelis-Menten equations [33] and diffusion rates. The thermodynamic and kinetic constants required to numerically solve the ODEs set were found in the specialized articles on AHL QS systems, in databases like BRENDA and B10NUMB3R5, or estimated as educated guesses. Further details can be found in [6].

As shown in Fig. 5 two biochemical steps takes place in the synthetic cell, namely, ① the production of a synthase E, and ② the enzymatic reaction

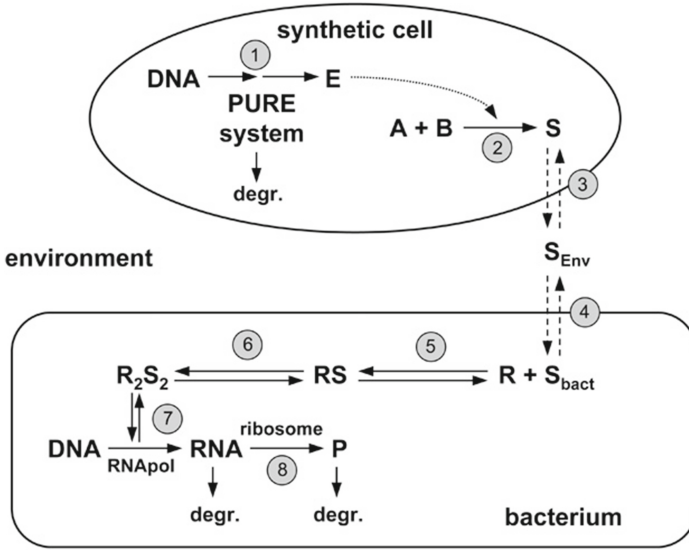


Fig. 5. Simplified model of synthetic cell sending a freely diffusible chemical signal (S) to a natural cell. Reproduced from [6] with the permission of Springer.

that produces S (the signal molecule) from the substrates A and B. These two processes are modelled by the ODEs nr. 1–2.

$$\frac{d[E]}{dt} = (k_{TXPst}) k_{TLPS} \cdot e^{-k_{inact}PSt} \tag{1}$$

$$\frac{d[A]}{dt} = \frac{d[B]}{dt} = -k_{cat}[E] \frac{[A]}{K_{MA} + [A]} \frac{[B]}{K_{MB} + [B]} \tag{2}$$

In particular, to model step ① we followed the experimentally derived profile obtained by the Yomo’s group (details in [34]), whereas the parameters reported by [35] were used to set up the Michaelis-Menten rate equation of step ②.

Two diffusion processes, modelled by ODEs nr. 3–4 and by the first term on the right-hand side of ODE nr. 5, bring the signal molecule from the sender nanomachine (the synthetic cell) to the receiver one (the bacterium). We have modelled the diffusive steps as simple gradient-driven permeation because the S molecule we have in mind (a short chain AHL) is considered a freely diffusible species [36]. The membrane permeability φ has been set to $0.1 \mu\text{m/s}$.

$$\frac{d[S]_{sc}}{dt} = k_{cat}[E] \frac{[A]}{K_{MA} + [A]} \frac{[B]}{K_{MB} + [B]} - \frac{\sigma_{sc}\varphi}{V_{sc}} ([S]_{sc} - [S]_{env}) \tag{3}$$

$$\frac{d[S]_{env}}{dt} = N_{sc} \frac{\sigma_{sc}\varphi}{V_{sc}} ([S]_{sc} - [S]_{env}) - N_{bact} \frac{\sigma_{bact}\varphi}{V_{bact}} ([S]_{env} - [S]_{bact}) \tag{4}$$

Reaching the bacterium by diffusion (process ④ in Fig. 5) the signal molecule S activates a series of steps, namely,

- ⑤ binding to the receptor R to give the non-covalent complex RS
- ⑥ non-covalent dimerization of RS to give the transcription factor R_2S_2
- ⑦ binding of R_2S_2 to the promoter region and activation of the transcription (operated by RNA polymerase)
- ⑧ translation of the messenger RNA produced in ⑦ to give the reporter protein P (operated by ribosomes)

By reporter protein we refer to a protein that can be easily detected in the bacterium, for example a fluorescent protein, or an enzyme catalyzing the formation of fluorescent or luminescent products. In this model, the reporter protein is 250 amino acid long. Moreover, two additional degradation reactions (messenger RNA and reporter protein) are considered. This reaction pattern is described by ODEs nr. 5–10

$$\frac{d[S]_{bact}}{dt} = \frac{\sigma_{bact}\phi}{V_{bact}} ([S]_{env} - [S]_{bact}) - k_{on}[R][S]_{bact} + k_{off}[RS] \quad (5)$$

$$\frac{d[R]}{dt} = -k_{on}[R][S]_{bact} + k_{off}[RS] \quad (6)$$

$$\frac{d[RS]}{dt} = k_{on}[R][S]_{bact} - k_{off}[RS] - 2k_{dim}[RS]^2 + 2k_{diss}[R_2S_2] \quad (7)$$

$$\frac{d[R_2S_2]}{dt} = k_{dim}[RS]^2 - k_{diss}[R_2S_2] \quad (8)$$

$$\frac{d[RNA]}{dt} = \frac{k_{TX}C_{RNApol}}{3L} \frac{C_{DNA}}{K_{DNA} + C_{DNA}} \frac{[R_2S_2]^n}{K_{R_2S_2}^n + [R_2S_2]^n} - k_{deg, RNA}[RNA] \quad (9)$$

$$\frac{d[P]}{dt} = \frac{k_{TL}C_{rib}}{L} \frac{[RNA]}{K_{RNA} + [RNA]} - k_{deg, P}[P] \quad (10)$$

A detailed discussion on how the about 40 parameters included in the model have been derived can be found in [6]. Most of them refer to *Escherichia coli*.

Simulation results are shown Fig. 6. The enzyme production inside the synthetic cell reaches a value of about $1 \mu\text{M}$, in good agreement with the experimental findings for PURE system TX-TL reactions. Once synthesized, E catalyzes the combination of A and B to give S, the signal molecule. A peak of S inside synthetic cell (ca. $0.8 \mu\text{M}$) appears at about 2.5 h, due to the fact that S accumulates inside synthetic cell and is slowly released in the environment, which acts as a sink, determining a concentration of S of about $0.2 \mu\text{M}$.

The high affinity of the signal molecule for its receptor S ($K_d = 10^{-3} \mu\text{M}$) and the efficient dimerization ($K_{dimerization} = 10^3 \mu\text{M}^{-1}$) bring about a ready formation of the transcription factor R_2S_2 . Its binding to the promoter has been described as cooperative, with an estimated Hill coefficient of 1.5 (cf. Eq. 9). Following these preliminary steps, transcription and translation finally start, producing in sigmoidal fashion both messenger RNA and reporter protein. A plateau [P] value of about $0.4 \mu\text{M}$ is reached after 4 h.

Although the outcomes of this simulation study actually encourage us to proceed with the experimental approach, these results are based on some parameters

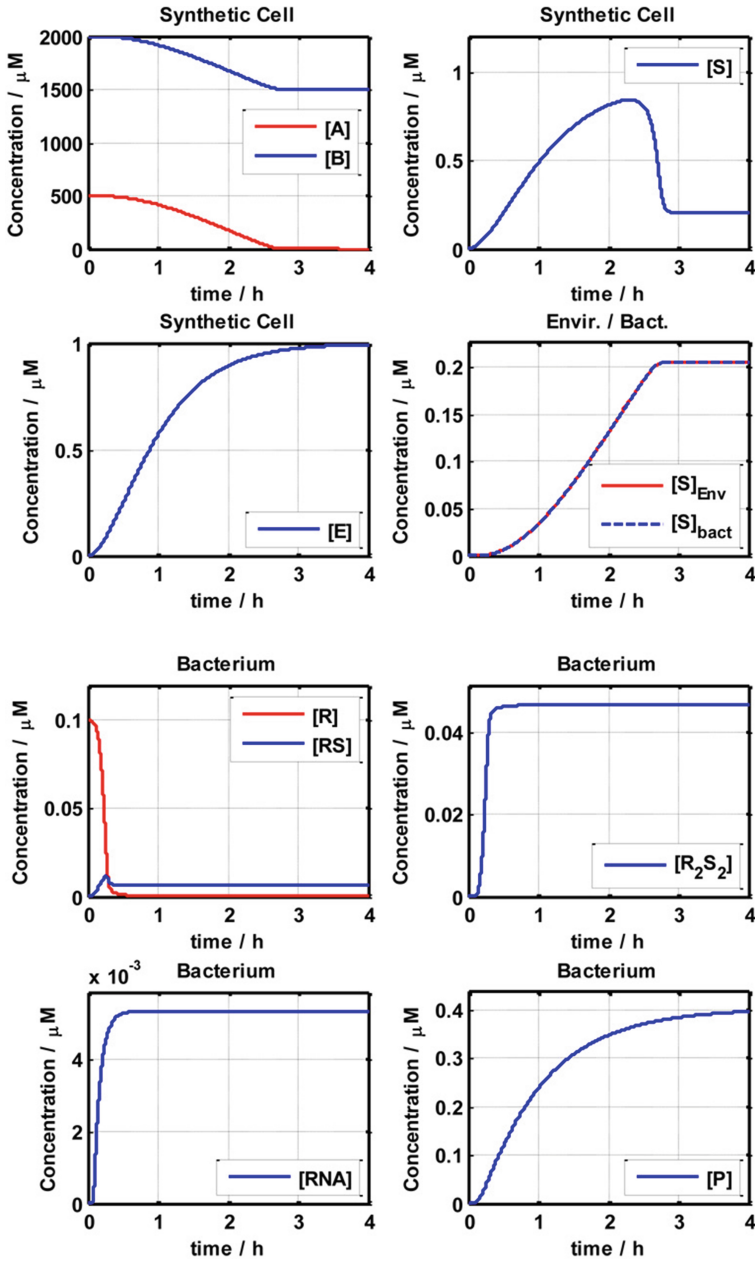


Fig. 6. Results of numerical integration (the ODEs nr. 1–10). Reproduced from [6] with the permission of Springer.

that have been estimated. In the next section, we will show what would be the result, in terms of kinetic and amount of reporter protein production, when these critical parameters are changed within a range of values. A positive outcome is considered only when a detectable amount of reporter protein is produced by the bacteria (considering a detection limit of around $0.05 \mu\text{M}$).

4.1 Parameters Scan

In order to assay whether the conclusions obtained by the model are robust with respect to our choices of its kinetic/thermodynamic parameters, we carried out a study on how the production of the reporter protein is affected by the parameter estimated values. This was done by keeping constant all parameters found in previous section (reported in [6]) and change only one of them. In particular, we focused on the following estimated parameters: (i) the permeability φ of the signal molecule across the lipid bilayer; (ii) the initial concentration of the receptor R in the bacterium ($[\text{R}_0]$); (iii) the efficiency of RS formation ($\text{R} + \text{S} \rightleftharpoons \text{RS}$), by changing the association rate constant (k_{on}); (iv) the dimerization efficiency ($2 \text{RS} \rightleftharpoons \text{R}_2\text{S}_2$) by changing the dimerization rate constant (k_{dim}).

The permeability φ strongly affects the timing of bacterial response, as shown in Fig. 7. When varied from 10^{-6} to 10^{-10} cm/s several effects are clearly visible in the dynamics of the receiving system. In particular, the formation of RS, and therefore of R_2S_2 , occurs at different time scales and in the case of extremely low φ (10^{-10} cm/s) the amount of R_2S_2 is limited to about 1 nM. Nevertheless, in all cases, the transcription is activated in a sigmoidal fashion, essentially because of the expected high affinity of R_2S_2 for the promoter region on the DNA (~ 25 pM). However, the RNA and protein syntheses are delayed more and more as the permeability coefficient decreases. Despite this, in all cases it results that bacteria produce an amount of reporter protein higher than $0.05 \mu\text{M}$ (the estimated threshold value) within about 2 h.

In contrast to the values of φ , scanning by orders of magnitude the (ii–iv) parameters does not affect significantly the production of the reporter protein, as illustrated in Figs. 8, 9 and 10. In all cases a variation of RS and R_2S_2 concentration is clearly evident, but this does not suffice to significantly change the bacterial transcription, and consequently the bacterial translation. For example, the amount of the receptor R in the bacterium could be much less than what we supposed to be ($0.1 \mu\text{M}$). In this case, the RS and R_2S_2 concentrations will be correspondingly reduced, but such a change will affect the TX-TL reaction in negligible way. The variation of the other parameters can be described accordingly.

From these results it is evident that even order-of-magnitude variations in the estimated parameters (φ , $[\text{R}]_0$, k_{on} , k_{dim}) do not significantly change the main conclusions that can be drawn by observing Fig. 6, with the exception of a significant timing effect when φ is strongly reduced. The protein synthesis is mainly controlled by the rate of transcription, which – in our model – is hardly affected by variations of R_2S_2 concentration, at least in the range of explored

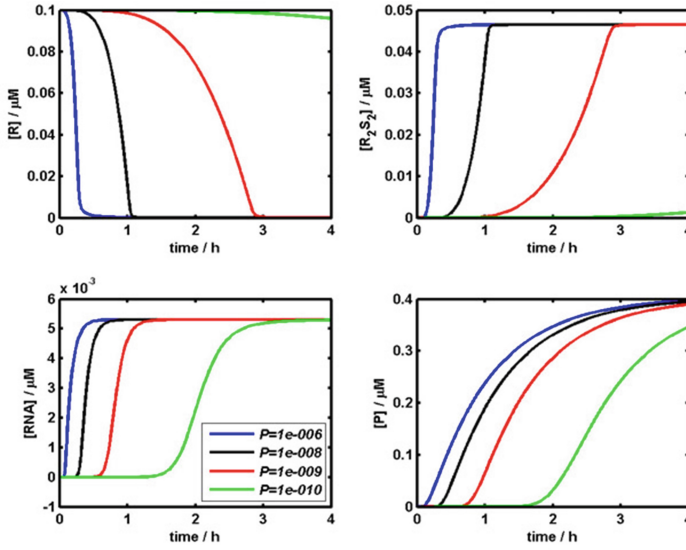


Fig. 7. Variations of bacterial response with the permeability coefficient (φ , cm/s): value in the model 10^{-5} ; scan from 10^{-6} to 10^{-10} .

parameters. The influence of the permeability constant is actually more related to physical reasons than to the biochemistry of the response circuitry. In order to better evidence this behaviour we plotted the rates of the processes occurring in our system against time (Fig. 11).

The rates of enzyme and signal molecule production inside synthetic cell are both high, do not change very much in time, and strongly correlate with each other. The rate of S release in the environment is somehow related to the rate of signal production in the synthetic cell, but it more than 100 times slower. It increases in time as S is synthesized, according to the generation of a larger chemical gradient across the synthetic cell membrane. On the other hand, as expected, the rate of R_2S_2 production, transcription and translation are correlated with each other, and the last two process in a very close manner (see arrows in Fig. 11). At short time, these three rates are all very low, but afterwards rapidly increases all together. Their behaviour diverges at long times because of the interplay of different reactions (mRNA and P degradation).

In conclusion, although our model is based on the estimation of several unknown kinetic and thermodynamic parameters, it results quite robust against changes of these estimates, mainly because of the estimated very high affinity of the transcription factor R_2S_2 for the DNA promoter. The permeability of the signal molecule is also a key factor determining the appearance of the reporter protein in the receiver cell (bacterium).

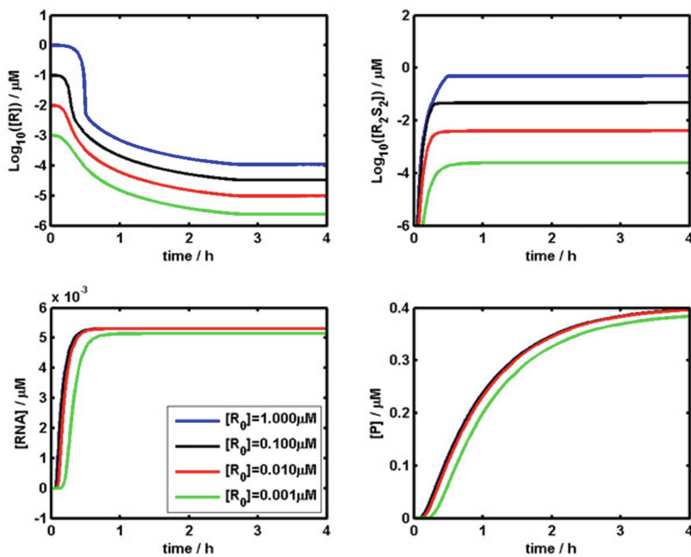


Fig. 8. Variations of bacterial response with the receptor R initial concentration ($[R_0]$, μM): value in the model 10^{-1} ; scan from 10^{-3} to $10^0 \mu\text{M}$.

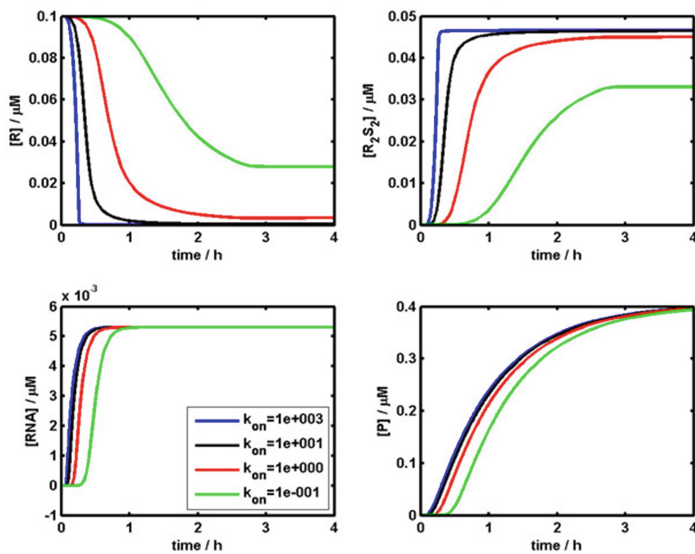


Fig. 9. Variations of bacterial response with the rate constant of RS formation (k_{on} , $\mu\text{M}^{-1} \text{s}^{-1}$): value in the model 10^2 ; scan from 10^3 to 10^{-1} .

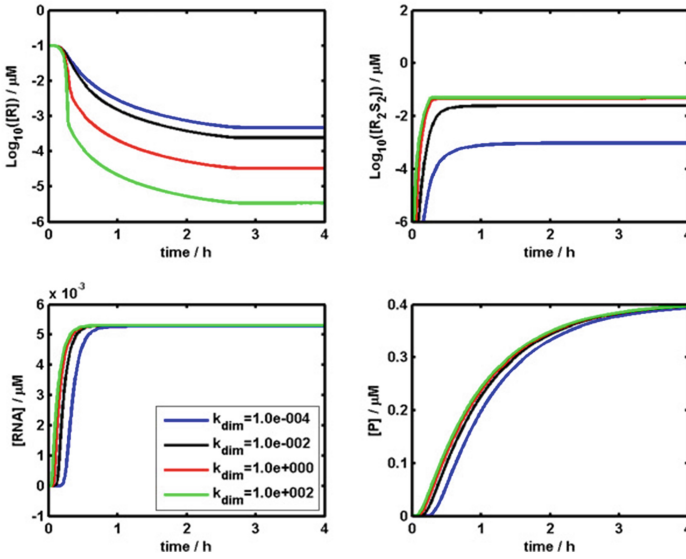


Fig. 10. Variations of bacterial response with the RS dimerization rate constant (k_{dim} , $\mu\text{M}^{-1} \text{s}^{-1}$): value in the model 10^0 ; scan from 10^{-4} to $10^2 \mu\text{M}^{-1} \text{s}^{-1}$

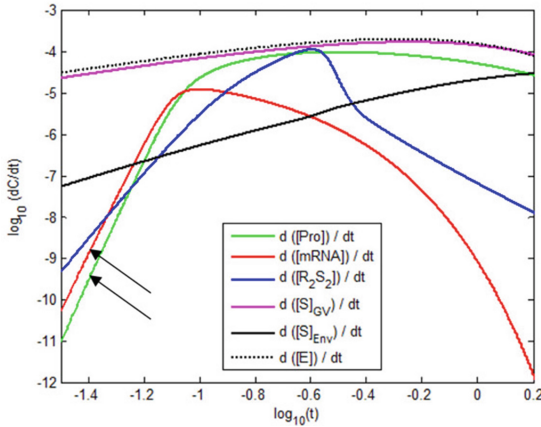


Fig. 11. Rates versus time plot referred to some processes of Fig. 5. Note the bilogarithmic scale.

5 Including the Spatial Information

Although it has not been explicitly said, the diffusion of S molecule is considered and modelled as only occurring across the membranes of synthetic cells and of bacteria, *i.e.*, the diffusion *through* the environment is considered as instantaneous. The model is built as a well-stirred reactor, meaning that – within each of the three compartments (synthetic cell, environment, bacteria) – the

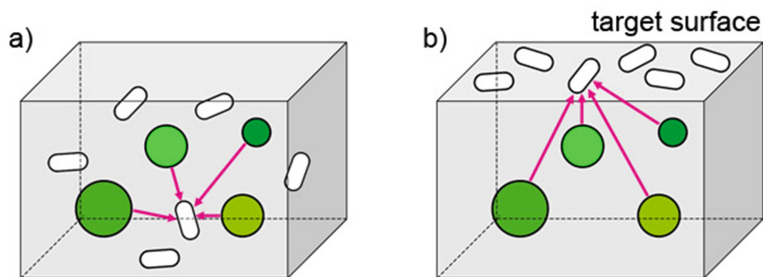


Fig. 12. Two possible way of let synthetic cells (green) and bacteria (white) interact by exchanging chemical signals, for example by a signal molecule sent by synthetic cells to bacteria. Different green tones describe difference of signal producing rate by synthetic cells. (a) Synthetic cells and bacteria are mixed; (b) synthetic cells and bacteria are kept separated. The pink arrows show which are the senders that contribute to deliver the signal molecule to a certain bacterium (Color figure online).

concentration of all species is instantaneously averaged in space, and the positions of the sender and receiver systems are not needed to run the simulations.

On the other hand, it is evident that from the practical viewpoint, different geometries can be realized, as those reported in Fig. 12. Essentially, synthetic cells and bacteria can be either mixed (Fig. 12a) or kept separated (Fig. 12b). In the first case, the two populations are disposed in 3D space as two interpenetrated arrays and the average distance between each synthetic cell and each bacterium is short.

In the second case, the two populations occupy two physically distinct regions of space, and these can be both 3D or 2D/3D (as shown in Fig. 12b). Here, the interface between the two regions is a sort of target surface for the sent molecules S , because the first receivers – those exposed to higher concentration of S – are going to respond efficiently.

A quantitative treatment of signal molecule diffusion in these two cases lies outside the scope of this article since it would require to treat the concentration of signal molecules as dependent both on time and spatial coordinates. In fact, even if vesicles and bacteria are assumed immobile (for example, embedded in a gel [37]) and dimensionless source and sink of signal molecules, respectively, the number of signal molecules that reach a certain target bacterium in a certain time results from the diffusion of such molecules from all synthetic cells present in the medium, and it depends from their spatial distribution, relative distance, and volume density. The model would give information about the response pattern of a population of bacteria exposed to a population of signal-emitting synthetic cells, for example according to the two scenarios proposed in Fig. 12. Based on random or non-random spatial distribution of senders and receivers, these calculations could be helpful to better define the most valuable experimental approaches.

6 Concluding Remarks

Synthetic cell technology, based on a bottom-up SB design, can be adapted and extended for reaching the ambitious goal of interfacing with biological cells, and this is possible by letting synthetic cells share with biological cells a common (chemical) language. These developments are realistic, since synthetic cells, due to their embodied molecular computing capabilities, would share with natural cells many abilities, such as chemical communication, molecular recognition, and signal transduction *i.e.*, the key features for reciprocal interfacing. Here we have presented a mathematical model that simulates the behaviour of synthetic cells sending a chemical signal to biological cells. It is, in essence, a useful tool for the construction of a synthetic-to-natural communication system based on a synthetic biology approach. It will be tested, optimized, modified and refined as soon as experimental data become available. It integrates the wet-lab approach with quantitative evaluations and/or order-of-magnitude estimates, which can be very helpful for designing the experiments in the proper way. The model has two main limitations: the first one is that it is a deterministic model, whereas stochastic effects are pervasive in small-scale systems [31,38]. Next, the spatial information about the diffusion of signal molecules has not been included in this first model. The generation of more realistic models where these two features are taken into account is the challenge for next steps in mathematical modelling synthetic cell/natural cell chemical communication.

More in general, the establishment of a molecular communication technology based on synthetic cells would be a cutting-edge technology for future advancements in practical and theoretical fields. From the practical viewpoint it will pave the way to the construction of soft-wet-micro-robotic systems with implications in nanomedicine and other applications, like bioremediation. From the theoretical viewpoint this will bring to novel strategies for natural computing in the bio-chem-ICTs arena, and possibly establish a novel paradigm for (embodied) artificial intelligence. Moreover, such a tool can be used to investigate basic scientific questions related to the mechanisms of cell signalling taking advantage of operating without the interference of background processes.

Addendum. A report on how synthetic cells can act as “translators” for natural cells has appeared during the preparation of this manuscript. In particular, synthetic cells have been constructed so that they produce α -hemolysin (from internal TX-TL reactions) upon addition of a trigger (theophylline). The consequent formation of a membrane pore (*cf.* with Fig. 4) allows the emission of a previously entrapped molecule (IPTG) that stimulates the bacterial response [39].

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