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A systems-theoretic model of a biological circuit for molecular communication in nanonetworks

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ABSTRACT

Recent advances in synthetic biology, in particular towards the engineering of DNA-based circuits, are providing tools to program man-designed functions within biological cells, thus paving the way for the realization of biological nanoscale devices, known as nanomachines. By stemming from the way biological cells communicate in the nature, Molecular Communication (MC), i.e., the exchange of information through the emission, propagation, and reception of molecules, has been identified as the key paradigm to interconnect these biological nanomachines into nanoscale networks, or nanonetwork. The design of MC nanonetworks built upon biological circuits is particularly interesting since cells possess many of the elements required to realize this type of communication, thus enabling the design of cooperative functions in the biological environment. In this paper, a systems-theoretic modeling is realized by analyzing a minimal subset of biological circuit elements necessary to be included in an MC nanonetwork design where the message-bearing molecules are propagated via free diffusion between two cells. The obtained system-theoretic models stem from the biochemical processes underlying cell-to-cell MC, and are analytically characterized by their transfer functions, attenuation and delay experienced by an information signal exchanged by the communicating cells. Numerical results are presented to evaluate the obtained analytical expressions as functions of realistic biological parameters.

1. Introduction

One of the goals of synthetic biology is the development of tools to reliably design and implement artificial functions within biological cells, thus enabling the realization of programmable biological nanoscale devices, known as biological nanomachines [2], based on living organisms, such as bacteria [21]. In particular, a synthetic biological circuit [19], or simply biological circuit, allows to program logical functions from simple controlled production of specific types of protein molecules, to complete engineered cell-to-cell interactions [18], in a similar way as it is done with electrical circuits. Molecular Communication (MC), defined as the exchange of information through the emission, propagation, and reception of molecules, has been identified as the key paradigm to interconnect these biological nanomachines into nanoscale networks, or nanonetwork. The design of MC nanonetworks built upon biological circuits is particularly interesting since cells possess many of the elements required to realize this type of communication, thus enabling the design of cooperative functions in the biological environment. The potential applications of this type of networks range from the biomedical [12], to the industrial and surveillance fields [23]. The focus of this paper is on the study from the communication engineering point of view of a molecular communication system based on biological circuits where the message-bearing molecules are propagated via free diffusion

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between two genetically-engineered cells, a transmitter and a receiver.

A biological circuit is normally defined as a genetic regulatory network [19] embedded in a biological cell, where DNA genes are linked together by activation and repression mechanisms that regulate their expression into proteins, which are biological macromolecules. Each DNA gene contains coding sequences, which are chemical information for building proteins, and regulatory sequences, which are sites were proteins can bind and control the rate of the gene expression, either by increasing (activation) or decreasing (repression) the protein building rate. In biological circuits, genes are interconnected such as the proteins produced by one or more genes regulate the expression of one or more genes. In recent years, a great effort is being devoted to the standardization and the establishment of catalogs of biological circuit parts [7]. By following the BioBrick™ standard [5], the units to measure the input and the output of a biological circuit are defined as Polymerases Per Second (PoPS), which correspond to the rates of the transcription process of the first and last biological circuit genes, respectively, proportional to their rate of expression. A biological circuit can process a PoPS signal as a function of the time in input by returning in output another PoPS signal as a function of the time through the aforementioned interconnection of gene regulations.

Some recent literature can be found on the analytical modeling of biological circuits, but with no specific mention to diffusion-based cell-to-cell communication through molecule exchange, for which only a biological description is provided in some specific works. Notable examples from this literature are given as follows. In [19] the genetic circuit design is introduced as an engineering discipline and the main mathematical framework for the modeling of biological circuit functions is introduced. The models of some important biological circuit patterns, called network motifs, are presented in a very complete theoretical framework in [3]. The standardization efforts of biological circuit parts are reviewed in [5], while the modeling techniques for biological circuits are discussed in [4]. The frequency domain analysis of biological circuits is presented in [8] both from a deterministic and a stochastic point of view, while the noise in biological circuit is discussed in [22]. In [16], the specific noise sources affecting cellular signaling pathways are described. Finally, the work in [21] treats engineering techniques to implement signals and sensors in bacteria through biological circuits.

In this paper, a systems-theoretic communication engineering model is presented for a biological circuit where a signal is transmitted from a PoPS input in a biological cell (transmitter cell) to a PoPS output in another biological cell (receiver cell), located at a predefined distance from the transmitter cell. This biological circuit, inspired by the cell-to-cell communication circuit sketched in [19], realizes a diffusion-based molecular communication system by encoding the signal to be transmitted into signaling molecules, which propagate between the transmitter cell and the receiver cell through their diffusion in the intercellular space. In addition, the biological circuit detailed in this paper is composed by the minimal subset of elements necessary to realize diffusion-based molecular communication between biological cells, and the resulting models are expected to have a general validity over other more complex implementations.

This paper is organized as follows. In Section 2 a biological circuit for molecular communication is identified through a minimal subset of elements. In Section 3 a systems-theoretic model is detailed in terms of transfer functions, from which analytical expressions are derived for the attenuation and the delay experienced by an information signal through the biological circuit. In Section 4 we present some numerical results obtained by applying to the developed models some realistic biological parameters from the literature. Finally, in Section 5 we conclude the paper.

2. A biological circuit for molecular communication

2.1. Functional blocks description

The main functional blocks of this biological circuit are shown in Fig. 1, where a space is divided into the intracellular environments of a transmitter cell and a receiver cell, respectively, which are assumed chemically homogeneous, or well-stirred, and they are divided by an intercellular environment. As a consequence, in the intracellular environment the molecule concentrations are assumed homogeneous in the space, while in the intercellular environment there is in general a non-homogeneous concentration of signaling molecules, which is subject to propagation via diffusion. We assume that the intracellular space of the transmitter cell is a volume with size Ω_{Tx}, while the intracellular space of the receiver cell is a volume with size Ω_{Rx}. The main functional blocks of this biological circuit, shown in Fig. 1, are detailed as follows:

- The *Signaling Enzyme Expression* takes place inside the transmitter cell, and it is initiated by a PoPS signal in input, $PoPS_{in}$, which promotes the transcription of an *enzyme coding sequence* and the translation of the contained information into a protein, denoted by E and called *enzyme* because of its specific chemical function, as explained in the following. The output of the signaling enzyme expression is the concentration of the produced enzymes, denoted by $[E]$.
- The *Signaling Molecule Production* is an enzymatic chemical reaction that occurs inside the transmitter cell, where the enzymes E catalyze the conversion of molecules present in the *intracellular environment*, called *substrates*, into other molecules, called products, by forming *enzyme–substrate complexes*. Among these products, the signaling molecules, denoted by S, are small organic molecules whose size allows them to cross the cell membrane and propagate through diffusion in the *intercellular environment*. The other products of the enzymatic reaction, denoted here as *subproducts*, remain in the intracellular environment and do not take part in the diffusion-based molecular communication. As a consequence, the input of the signaling molecule production is the concentration of enzymes $[E]$, while the output is the concentration of produced signaling molecules at the transmitter, denoted by $[S]_{Tx}$.

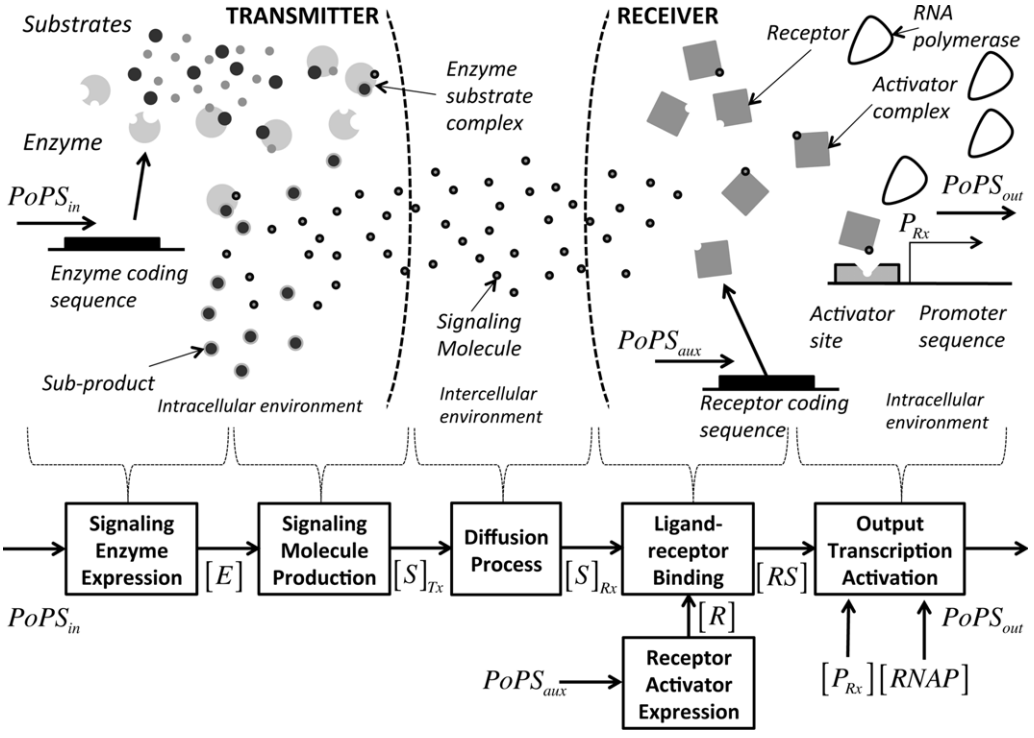

Fig. 1. Main functional blocks of a biological circuit for diffusion-based molecular communication.

- The *Diffusion Process* realizes the propagation of the signaling molecules S in the intercellular environment, and it is the macroscopic effect of the random Brownian motion of the signaling molecules in the space. The diffusion process has the effect to propagate differences in the signaling molecule concentration from the transmitter cell to the receiver cell, where they cross the membrane and have access to the receiver intracellular environment. The input of the diffusion process is the concentration $[S]_{Tx}$ of signaling molecules at the transmitter cell, while the output is the concentration $[S]_{Rx}$ of signaling molecules at the receiver cell.
- The *Receptor Activator Expression* takes place inside the receiver cell, and it is initiated by an input PoPS auxiliary signal, $PoPS_{aux}$, which promotes the transcription of a *receptor coding sequence* and the translation of the contained information into proteins, called receptors, and denoted by R. The output of the receptor activator expression is the concentration of the produced receptors, denoted by $[R]$.
- The *Ligand–Receptor Binding* is a reaction that occurs inside the receiver cell, where the incoming signaling molecules S bind to the receptors R and form activator complexes, denoted by RS. The inputs of the ligand–receptor binding are the concentration of produced receptors $[R]$ and the concentration $[S]_{Rx}$ of signaling molecules at the receiver cell, and the output is the concentration $[RS]$ of activator complexes.
- The *Output Transcription Activation* is initiated by the activator complexes RS upon binding to the *activator site*, where a PoPS output signal is produced according to the binding of RNA polymerase proteins, denoted as *RNAP*, to the promoter sequence. The inputs of the transcription activation are the concentration $[RS]$ of activator complexes, the concentration $[P_{Rx}]$ of promoter sequences, and the concentration $[RNAP]$ of the RNA polymerase protein, respectively, while the output PoPS signal is denoted as $PoPS_{out}$.

2.2. Reaction-based description

In the following, we provide a description of the biological circuit in terms of the chemical reactions undergoing in the aforementioned elements. This description serves to define all the chemical parameters of the biological circuit under study, and it sets the basis to build the systems-theoretic model detailed in Section 3.

The *Signaling Enzyme Expression* is based on a *transcription and translation reaction*, which models the production of the np_E enzymes stimulated by the input signal $PoPS_{in}$ with a rate k_E, expressed as follows:

$$PoPS_{in} \xrightarrow{k_E} np_E E + PoPS_{in}. \tag{1}$$

The enzymes are also subject to degradation, with a degradation rate k_{d_E}, expressed as:

$$E \xrightarrow{k_{d_E}} (). \tag{2}$$

The *Signaling Molecule Production* is based on an enzymatic reaction where the enzyme E and the substrates (one or more), here denoted as S_0, according to the rate k_{S_1} form a complex C_s, which can then either dissociate back into the enzyme E and the substrates S_0, with a rate $k_{S_{-1}}$, or evolve

into the sum of the enzyme E and the signaling molecule S according to a rate k_{S_2}. This reaction is expressed as follows:

$$E + S_0 \underset{k_{S_{-1}}}{\overset{k_{S_1}}{\longleftrightarrow}} C_S \xrightarrow{k_{S_2}} E + S. \tag{3}$$

The *Diffusion Process* is based on the assumption to have a 3-dimensional intercellular space, which contains a fluidic medium and has infinite extent in all the three dimensions. The diffusion process is based on the following *Diffusion Equation* [20,9] in the variable $[S](r, t)$, which is the concentration $[S]$ of signaling molecules present at distance r from the transmitter and time instant t:

$$\frac{\partial [S](r,t)}{\partial t} = D\nabla^2 [S](r,t), \tag{4}$$

where $\partial(\cdot)/\partial t$ and $\nabla^2(\cdot)$ are the time first derivative and the Laplacian operator, respectively. D is the diffusion coefficient and it is considered a constant parameter within the scope of this paper. This is in agreement with the assumption of having independent Brownian motion for every molecule in the space.

The *Receptor Activator Expression* is based on the *transcription and translation reaction* for the production of np_R receptors R stimulated by the signal $PoPS_{aux}$ with a rate k_R, expressed as follows:

$$PoPS_{aux} \xrightarrow{k_R} np_R R + PoPS_{aux}. \tag{5}$$

The degradation reaction of the receptors R is expressed as follows according to a degradation rate k_{d_R}:

$$R \xrightarrow{k_{d_R}} (). \tag{6}$$

The *Ligand–Receptor Binding* is based on the binding and release reactions between receptors R and signaling molecules S. Upon binding, which occurs with a rate k_{RS}, a receptor R and a signaling molecule S form an activator complex RS, which will be the input of the next transcription activation reaction. A complex RS unbinds and releases a receptor R and a signaling molecule S according to a rate k_{-RS}. This is expressed as follows:

$$R + S \underset{k_{-RS}}{\overset{k_{RS}}{\longleftrightarrow}} RS. \tag{7}$$

The *Output Transcription Activation* is based on an *open complex formation reaction*, where an activator complex RS, a promoter sequence P_{Rx}, and an RNA polymerase $RNAP$ trigger the open complex formation, quantified through the output signal $PoPS_{out}$, according to a rate k_{Rx}. The open complex can dissociate back into an activator complex RS, a promoter sequence P_{Rx}, and an RNA polymerase $RNAP$ according to a rate k_{-Rx}. This has the following expression:

$$RS + P_{Rx} + RNAP \underset{k_{-Rx}}{\overset{k_{Rx}}{\longleftrightarrow}} PoPS_{out}. \tag{8}$$

In the following, with reference to the aforementioned chemical reactions, we detail the systems-theoretic model of this biological circuit, which allows to derive the transfer function and, consequently, the attenuation and delay parameters for each functional block and for their overall end-to-end cascade.

3. Systems-theoretic model

The objective of the systems-theoretic model is to derive the mathematical relation between the input signal $PoPS_{in}(t)$ and the output signal $PoPS_{out}(t)$ of the aforementioned biological circuit for diffusion-based molecular communication, where the input and output signals are function of the time t. As detailed in the following, we express this mathematical relation in terms of transfer function $H(\omega)$, where ω corresponds to the frequency of the Fourier transforms [10] of the signals, namely, $\mathbf{PoPS_{in}}(\omega)$ and $\mathbf{PoPS_{out}}(\omega)$, expressed as follows:

$$\begin{aligned} H(\omega) &= \frac{\mathbf{PoPS_{out}}(\omega)}{\mathbf{PoPS_{in}}(\omega)}, \\ \mathbf{PoPS_i}(\omega) &= \int PoPS_{\mathbf{i}}(t) e^{-j\omega t} dt, \end{aligned} \tag{9}$$

where $\mathbf{i} \in \{in, out\}$, and $H(\omega)$ depends from all the chemical parameters defined in Section 2, namely, the transmitter cell volume Ω_{Tx} and the receiver cell volume Ω_{Rx}, the reaction rates k_E, k_{d_E}, k_{S_1}, $k_{S_{-1}}$, k_{S_2}, k_R, k_{d_R}, k_{RS}, k_{-RS}, k_{Rx}, k_{-Rx} and numbers of produced molecules np_E and np_R, the diffusion coefficient D, the auxiliary signal $PoPS_{aux}$, assumed constant in time, the concentration of substrates $[S_0]$ at the transmitter cell, and the concentrations of promoter sequence $[P_{Rx}]$ and RNA polymerase $[RNAP]$ at the receiver cell.

As explained in the following and graphically shown in Fig. 2, the linearity property of the mathematical expressions of the chemical reactions described in Section 2.2 allows the decomposition of the transfer function $H(\omega)$ into the cascade of the transfer functions of each functional block, as shown in Fig. 1. This decomposition has the following expression:

$$\begin{aligned} H(\omega) \;=\; & H_A(\omega) H_B(\omega) H_C(\omega) H_D(\omega) H_E(\omega) H_F(\omega) [S_0] \\ & \cdot PoPS_{aux} [P_{Rx}] [RNAP], \end{aligned} \tag{10}$$

where $H_X(\omega)$, $X \in \{A, B, C, D, E, F\}$, are the transfer functions of each functional block, as function of the frequency ω, detailed in the following. The parameters $[S_0]$, $PoPS_{aux}$, $[P_{Rx}]$ and $[RNAP]$ are the concentration of substrates at the transmitter cell, and the auxiliary input signal, the concentration of promoter sequences and the concentration of RNA polymerase at the receiver cell, respectively, assumed constant in time for the scope of this paper.

In the following, we analytically derive the transfer function of each functional block shown in Fig. 2 from the reaction-based description provided in Section 2.2. Subsequently, we provide an approximation $\hat{H}(\omega)$ of the transfer function of the biological circuit through considerations on the differences in the time scales of the chemical reactions of different functional blocks. Finally, starting from the expression of the approximated transfer function $\hat{H}(\omega)$ of the biological circuit, we provide analytical expressions for the attenuation and delay experienced by an information signal through the biological circuit.

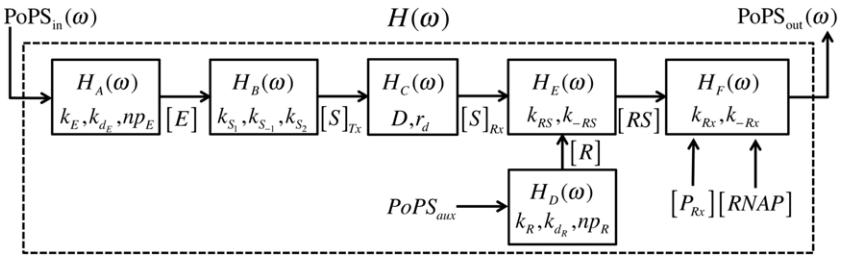

Fig. 2. Decomposition of the transfer function of a biological circuit for diffusion-based molecular communication into the transfer functions of each functional block.

3.1. Functional block transfer functions

The transfer function of each functional block, with reference to Fig. 2, is analytically derived by applying the Classical Chemical Kinetic (CCK) modeling [6] to the reaction-based description provided for each block in Section 2.2.

The CCK model of the *Signaling Enzyme Expression* is expressed through the following Reaction-Rate Equation (RRE), which analytically models the chemical reactions in (1) and (2):

$$\frac{d[E](t)}{dt} = np_E k_E PoPS_{in}(t) - k_{d_E}[E](t), \tag{11}$$

where $[E](t)$ and $PoPS_{in}(t)$ are the concentration of produced enzymes inside the transmitter cell and the input signal, respectively, as functions of the time t. By applying the Fourier transform [10] to (11), we obtain the following:

$$j\omega[\mathbf{E}](\omega) = np_E k_E \mathbf{PoPS_{in}}(\omega) + k_{d_E}[\mathbf{E}](\omega). \tag{12}$$

As a consequence, the transfer function $H_A(\omega)$ of the signaling enzyme expression functional block is derived by solving (12) with respect to the concentration of produced enzymes $[\mathbf{E}](\omega)$ as function of the $\mathbf{PoPS_{in}}(\omega)$, expressed as

$$H_A(\omega) = \frac{np_E k_E}{j\omega + k_{d_E}}, \tag{13}$$

where np_E, k_E, and k_{d_E} are the number of enzymes produced per reaction, the enzyme expression rate, and the enzyme degradation rate, respectively.

The *Signaling Molecule Production* is expressed through the following two RREs, which analytically model the chemical reactions in (3):

$$\frac{d[C_S](t)}{dt} = k_{S_1}[E](t)[S_0] - k_{S_{-1}}[C_S](t) - k_{S_2}[C_S](t)$$
$$\frac{d[S]_{Tx}(t)}{dt} = k_{S_2}[C_S](t), \tag{14}$$

where $[C_S](t)$, $[E](t)$, and $[S]_{Tx}(t)$ are the concentration of formed complexes, produced enzymes and produced signaling molecules inside the transmitter cell, respectively, as functions of the time t, and $[S_0]$ is the concentration of the substrates, assumed constant in time. By applying the Fourier transform [10] to (14) and by substituting the first expression in the second expression, we obtain

$$j\omega[\mathbf{S}]_{\mathbf{Tx}}(\omega) = \frac{k_{S_1}}{k_{S_{-1}} + k_{S_2} + j\omega}[S_0][\mathbf{E}](\omega). \tag{15}$$

Starting from (15), by expressing the concentration of produced signaling molecules $[\mathbf{S}]_{\mathbf{Tx}}(\omega)$ as function of the produced enzymes $[\mathbf{E}](\omega)$ and the frequency ω, we derive the expression of the transfer function $H_B(\omega)$ of the signaling molecule production functional block as follows:

$$H_B(\omega) = \frac{k_{S_1}}{\omega\{j(k_{S_{-1}} + k_{S_2}) - \omega\}}, \tag{16}$$

where k_{S_1}, $k_{S_{-1}}$, and k_{S_2} are the complex formation rate, the complex dissociation rate, and the signaling molecule production rate, respectively.

The *Diffusion Process* functional block is expressed through the Inhomogeneous Diffusion Equation, which is based on the diffusion equation expression in (4), as follows:

$$\frac{\partial[S](r,t)}{\partial t} = D\nabla^2[S](r,t) + \frac{d[S]_{Tx}(t)}{dt}\delta(r), \tag{17}$$

where $[S](r,t)$ and $\frac{d[S]_{Tx}(t)}{dt}$ are the concentration of signaling molecules present at distance r from the transmitter and the first time derivative of the concentration of signaling molecules at the transmitter, respectively, as function of the time t. $\delta(r)$ is a Dirac delta centered at the transmitter location and D is the diffusion coefficient. The solution of (17) in terms of Fourier transform [10] of the concentration of signaling molecules $[\mathbf{S}]_{\mathbf{Rx}}(\omega)$ at the receiver, located at a distance r_{Rx} from the transmitter, as function of the produced signaling molecules $[\mathbf{S}]_{\mathbf{Tx}}(\omega)$, is as follows [17]:

$$[\mathbf{S}]_{\mathbf{Rx}}(\omega) = \frac{e^{-(1+j)\sqrt{\frac{\omega}{2D}}r_{Rx}}}{\pi D r_{Rx}} j\omega[\mathbf{S}]_{\mathbf{Tx}}(\omega). \tag{18}$$

As a consequence, the expression of the transfer function $H_C(\omega)$ of the diffusion process functional block is as follows:

$$H_C(\omega) = j\omega\frac{e^{-(1+j)\sqrt{\frac{\omega}{2D}}r_{Rx}}}{\pi D r_{Rx}}, \tag{19}$$

where D and r_{Rx} are the diffusion coefficient and the distance of the receiver from the transmitter, respectively.

The CCK model of the *Receptor Activator Expression* is expressed through the following RRE, which analytically models the chemical reactions in (5) and (2):

$$\frac{d[R](t)}{dt} = np_R k_R PoPS_{aux} - k_{d_R}[R](t), \tag{20}$$

where $[R](t)$ and $PoPS_{aux}$ are the concentration of receptors inside the receiver cell as functions of the time t and the PoPS signal that controls the receptor expression, respectively. Since we assume that the auxiliary input signal $PoPS_{aux}$ is constant in time, the resulting concentration of receptors inside the receiver cell is also constant in time. By solving (20), the expression of the transfer function $H_D(\omega)$ of the diffusion process functional block is as follows:

$$H_D(\omega) = \frac{np_R k_R}{k_{d_R}}, \tag{21}$$

where np_R, k_R, and k_{d_R} are the number of receptors produced per reaction, the receptor expression rate, and the receptor degradation rate, respectively.

The *Ligand–Receptor Binding* has a RRE CCK model which derives from the chemical reaction expression in (7), and it is as follows:

$$\frac{d[RS](t)}{dt} = k_{RS}[R](t)[S]_{Rx}(t) - k_{-RS}[RS](t), \tag{22}$$

where $[RS](t)$, $[R](t)$ and $[S]_{Rx}(t)$ are the concentration of activator complexes, receptors and signaling molecules inside the receiver cell, respectively, as functions of the time t. By applying the Fourier transform [10] to (22), we express the concentration of activator complexes $[\mathbf{RS}](\omega)$ as function of $[\mathbf{R}](\omega)$, $[\mathbf{S}](\omega)$, and the frequency ω as follows:

$$j\omega[\mathbf{RS}](\omega) = ([\mathbf{R}](\omega) * [\mathbf{S}](\omega))\, k_{RS} - k_{-RS}[\mathbf{RS}](\omega), \tag{23}$$

where $. * .$ is the convolution operator [10]. As explained above, we assume a constant auxiliary input signal $PoPS_{aux}$ is constant in time, which results in a constant concentration of receptors inside the receiver cell. As a consequence, the expression of the transfer function $H_E(\omega)$ of the ligand–receptor binding functional block is as follows:

$$H_E(\omega) = \frac{k_{RS}}{j\omega + k_{-RS}}, \tag{24}$$

where k_{RS} and k_{-RS} are the ligand–receptor binding and release rates, respectively.

The CCK model of the *Output Transcription Activation* functional block is expressed through the RRE, which is derived from the description of the chemical reaction in (8). This RRE has the following expression:

$$\frac{dPoPS_{out}(t)}{dt} = k_{Rx}[P_{Rx}][RNAP][RS](t) - k_{-Rx}[RS](t), \tag{25}$$

where $PoPS_{out}(t)$ and $[RS](t)$ are the biological circuit output PoPS signal and the concentration of activator complexes inside the receiver cell, respectively, as functions of the time t, and $[P_{Rx}]$ and $[RNAP]$ are the concentrations of promoter sequences and RNA polymerase at the receiver cell, respectively, assumed constant in time. The expression in (22) is solved in the same way as done for the signaling enzyme expression functional block in (12). Finally, the expression of the transfer function $H_F(\omega)$ of the output transcription activation functional block is as follows:

$$H_F(\omega) = \frac{k_{Rx}}{j\omega + k_{-Rx}}, \tag{26}$$

where k_{Rx}, and k_{-Rx} are the open complex formation and dissociation rates, respectively.

Since the RRE expression of the functional blocks in (11), (14), (17), (20), and (22) are Ordinary Differential Equations (ODE), they represent Linear Time-Invariant systems, whose transfer function solutions can be combined through the formula in (10) to derive the transfer function $H(\omega)$ of a biological circuit for diffusion-based molecular communication, expressed as

$$\begin{aligned} H(\omega) = {} & \frac{np_E k_E}{j\omega + k_{d_E}} \frac{k_{S_1}[S_0]}{\omega\{j(k_{S_{-1}} + k_{S_2}) - \omega\}} \\ & \times j\omega \frac{e^{-(1+j)\sqrt{\frac{\omega}{2D}} r_{Rx}}}{\pi D r_{Rx}} \\ & \cdot \frac{np_R k_R}{k_{d_R}} PoPS_{aux} \frac{k_{RS}}{j\omega + k_{-RS}} \\ & \cdot \frac{k_{Rx}}{j\omega + k_{-Rx}} [P_{Rx}][RNAP], \end{aligned} \tag{27}$$

where np_E, k_E, and k_{d_E} are the number of enzymes produced per reaction, the enzyme expression rate, and the enzyme degradation rate, respectively, k_{S_1}, $k_{S_{-1}}$, and k_{S_2} are the complex formation rate, the complex dissociation rate, and the signaling molecule production rate, respectively, D and r_{Rx} are the diffusion coefficient and the distance of the receiver from the transmitter, respectively, np_R, k_R, and k_{d_R} are the number of receptors produced per reaction, the receptor expression rate, and the receptor degradation rate, respectively, $PoPS_{aux}$ is the auxiliary input signal, assumed constant in time, k_{RS} and k_{-RS} are the ligand–receptor binding and release rates, respectively, and k_{Rx}, and k_{-Rx} are the open complex formation and dissociation rates, respectively. $[S_0]$, $[P_{Rx}]$ and $[RNAP]$ are the concentrations of substrates at the transmitter cell, promoter sequences and RNA polymerase at the receiver cell, respectively, assumed constant in time.

3.2. Time scale approximation

According to [3], the chemical reactions involved in biological circuits have different time scales. In particular, the chemical reactions described in Section 2.2, and modeled through the transfer function expressions in Section 3.1, occur at significantly different speeds. As experimentally demonstrated in [3], the chemical reactions where a protein is expressed from the DNA coding sequence and accumulates/propagates in the space, such as the signaling enzyme expression, the receptor activator expression, and the diffusion process, are significantly slower than the reactions between two or more molecules for the formation of complexes, such as in the signaling molecule production, the ligand–receptor binding and the output transcription activation. Therefore, the former reactions dominate the dynamic behavior of the circuit, and the transfer functions of the latter reactions can be approximated with their steady state versions, as shown in Fig. 3 and analytically derived in the following.

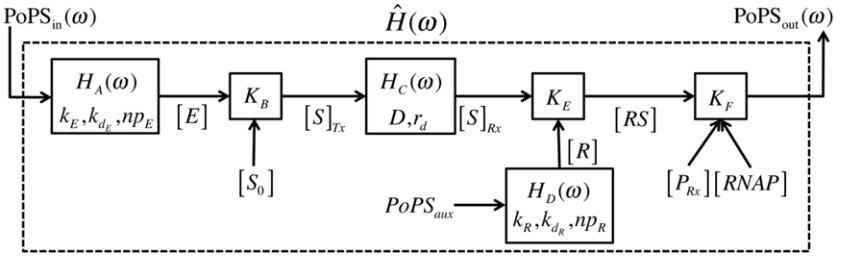

Fig. 3. Approximation of the decomposition of the transfer function of a biological circuit for diffusion-based molecular communication.

As a result, we define $\hat{H}(\omega)$ as the approximate transfer function of the biological circuit, derived through considerations on the chemical reaction time scales. The transfer function $\hat{H}(\omega)$ is expressed as follows:

$$\hat{H}(\omega) = H_A(\omega)K_B(\omega)[S_0]H_C(\omega)H_D(\omega) \cdot PoPS_{aux}K_EK_F[P_{Rx}][RNAP], \quad (28)$$

where $H_A(\omega)$, $H_C(\omega)$, and $H_D(\omega)$ are the transfer functions of the signaling enzyme expression, the receptor activator expression, and the diffusion process, respectively, $[S_0]$, $PoPS_{aux}$, $[P_{Rx}]$ and $[RNAP]$ are the concentrations of substrates at the transmitter cell, the auxiliary input, the concentration promoter sequences and the concentration RNA polymerase at the receiver cell, respectively, assumed constant in time, $K_B(\omega)$ is the steady state transfer function of the signaling molecule production, K_E, and K_F are the steady state approximations to constant values of the transfer functions of the ligand–receptor binding and the output transcription activation, respectively.

The steady state approximation $K_B(\omega)$ of the *Signaling Molecule Production* functional block is computed by setting in (14) the first time derivative $d[C_S](t)/dt$ in the concentration of formed complexes to 0. The solution to (14) becomes as follows:

$$\frac{d[C_S](t)}{dt} = 0 \rightarrow [\mathbf{S}]_{\mathbf{Tx}}(\omega) = \frac{k_{S_1}}{j\omega(k_{S_{-1}} + k_{S_2})}[S_0][\mathbf{E}](\omega). \quad (29)$$

The steady state transfer function $K_B(\omega)$ of the signaling molecule production is therefore given by

$$K_B(\omega) = \frac{k_{S_1}}{j\omega(k_{S_{-1}} + k_{S_2})}, \quad (30)$$

where k_{S_1}, $k_{S_{-1}}$, and k_{S_2} are the complex formation rate, the complex dissociation rate, and the signaling molecule production rate, respectively.

The steady state approximations of the *Ligand–Receptor Binding* and the *Output Transcription Activation* functional blocks to the constant values K_E and K_F result from computing the transfer functions $H_E(\omega)$ and $H_F(\omega)$ for a value of the frequency $\omega = 0$, expressed as

$$K_E = \frac{k_{RS}}{k_{-RS}}, \qquad K_F = \frac{k_{RX}}{k_{-RX}}, \quad (31)$$

which correspond to the solution of (22) and (25) when we set to 0 the time first derivative $d[RS](t)/dt$ in the concentration of activator complexes and the time first derivative $dPoPS_{out}(t)/dt$ in the biological circuit output PoPS signal.

The approximate transfer function $\hat{H}(\omega)$ of the biological circuit, derived through the steady state approximations, has the following expression:

$$\hat{H}(\omega) = K_{\hat{H}} \frac{e^{-(1+j)\sqrt{\frac{\omega}{2D}}r_{Rx}}}{j\omega(j\omega + k_{d_E})}, \quad (32)$$

where the constant $K_{\hat{H}}$ is as follows:

$$K_{\hat{H}} = \frac{np_E k_E k_{S_1}[S_0]np_R k_R PoPS_{aux} k_{RS} k_{Rx}[P_{Rx}][RNAP]}{(k_{S_{-1}} + k_{S_2})\pi D r_{Rx} k_{d_R} k_{-RS} k_{-Rx}}, \quad (33)$$

where all the parameters are the same as in (27).

3.3. Attenuation and delay expressions

The attenuation and delay experienced by a signal through the biological circuit are analytically derived from the approximate transfer function $\hat{H}(\omega)$ expressed in (32),

The attenuation $\alpha(\omega)$, as function of the frequency ω, is computed through the reciprocal of the absolute value of the approximate transfer function $\hat{H}(\omega)$ in (32), which has the following expression:

$$\alpha(\omega) = \frac{1}{|\hat{H}(\omega)|} = \frac{\omega\sqrt{\omega^2 + k_{d_E}^2}}{K_{\hat{H}} e^{-\sqrt{\frac{\omega}{2D}}r_{Rx}}}, \quad (34)$$

where k_{d_E} is the enzyme degradation rate, D is the diffusion coefficient, r_{Rx} is the distance of the receiver from the transmitter, and $K_{\hat{H}}$ is given in (33).

The delay $\Delta(\omega)$, as function of the frequency ω, is computed as the frequency first derivative of the phase $\phi_{\hat{H}}(\omega)$ of the approximate transfer function $\hat{H}(\omega)$ in (32). The phase $\phi_{\hat{H}}(\omega)$ has the following expression

$$\phi_{\hat{H}}(\omega) = \arctan\left(\frac{k_{d_E}}{\omega}\right) - \sqrt{\frac{\omega}{2D}}r_{Rx}. \quad (35)$$

As a consequence, the delay $\Delta(\omega)$ is expressed as

$$\Delta(\omega) = -\frac{d\phi_{\hat{H}}(\omega)}{d\omega} = \frac{r_{Rx}\omega^2 + 2k_{d_E}\sqrt{2D\omega} + r_{Rx}k_{d_E}^2}{2\sqrt{2D\omega}\left(\omega^2 + k_{d_E}^2\right)}, \quad (36)$$

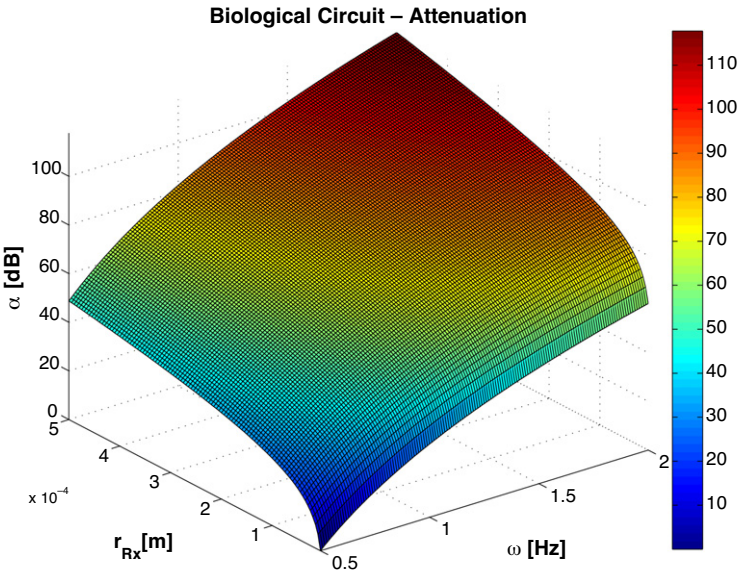

Fig. 4. Attenuation of the biological circuit for diffusion-based MC as function of the receiver distance from the transmitter r_{Rx} and the frequency ω.

where r_{Rx}, k_{d_E}, and D are the distance of the receiver from the transmitter, the enzyme degradation rate, and the diffusion coefficient, respectively.

4. Numerical results

In this section, we present some preliminary numerical results obtained through the evaluation of the expressions of the systems-theoretic model of the biological circuit for diffusion-based MC analyzed in this paper.

In the synthetic biology and biological circuit engineering literature, there are currently very few works that focus on the joint experimental determination of the biochemical parameters of a complete biological circuit implementation, such as the parameters we introduced in Section 2.2. Most of the results presented in the literature focus on the study of one specific element or biochemical reaction rather than a complete architecture. As a consequence, the values of the biochemical parameters used for our numerical results are taken from a diverse pool of papers and, although they satisfy the goal of having a realistic order of magnitude, they do not necessarily capture the values that they would have in a real implementation of the biological circuit we analyze here.

For the numerical results obtained from the system-theoretic model, presented in the following, we applied the following parameter values from the LuxR–LuxI quorum sensing system [13] in *E. coli* bacteria, which has been already used for the engineering of a biological circuit for diffusion-based molecular communication, as in [1]. The rates of signaling enzyme and receptor activator translation equal to the rate of Lux protein translation from [15], namely, $k_E = k_R = 9.6 \times 10^{-1}$ min^{-1}, the rate of signaling enzyme degradation equal to the degradation rate of LuxI protein in [15], namely, $k_{d_E} = 1.67 \times 10^{-2}$ min^{-1}, the rate of receptor degradation equal to the degradation rate of LuxR protein in [15], namely, $k_{d_R} = 2.31 \times 10^{-2}$ min^{-1}, the complex formation rate from the binding of the signaling enzymes and the substrates equal to the forward LuxI–substrates reaction for autoinducer molecule production in [14], namely, $k_{S_1} = 0.6$ molecules^{-1} min^{-1}, the binding and unbinding rates between receptors and signaling molecules equal to the values in [15], namely, $k_{RS} = 6 \times 10^{-4}$ molecules^{-1} min^{-1} and $k_{\_RS} = 2 \times 10^{-2}$ min^{-1}, respectively, and the rate of open complex formation upon output transcription activation and open complex dissociation at the receiver as in [15], namely, $k_{Rx} = 10^{-2}$ molecules^{-1} min^{-1} and $k_{\_Rx} = 4 \times 10^{-2}$ min^{-1}. The diffusion coefficient $D \sim 60 \times 10^{-9}$ m^2 min^{-1} is set to the diffusion coefficient of molecules diffusing in a biological environment (cellular cytoplasm, [11]). We set the volume of the transmitter and receiver volume $\Omega_{Tx} = \Omega_{Rx}$ to 1 μm^2, the number of substrates at the transmitter cell $S_0 = 100$, the auxiliary input signal $PoPS_{aux} = 1$, the number of promoter sequences and RNA polymerases at the receiver cell $P_{Rx} = 1$ and $RNAP = 100$, respectively.

In Figs. 4 and 5 we show the numerical results for the attenuation α and delay Δ experienced by a signal through the biological circuit, computed by using the expressions in (34) and (36), respectively. The value of the receiver distance from the transmitter r_{Rx} ranges from 5 to 500 μm, while the range of observed frequency ω values is between 0 and 1 Hz. The values for the attenuation α of the biological circuit, shown in dB, range from a minimum of 0 at the minimum distance r_{Rx} and frequency ω equal to 0, to a maximum of 117.8 dB for a distance r_{Rx} of 50 μm and frequency ω equal to 1 Hz with a monotonic increasing trend both when increasing the distance r_{Rx} and the frequency ω. The values for the delay Δ range from a minimum of 0 min at the minimum distance r_{Rx} and maximum frequency ω equal to 1 Hz, to a maximum of 1.4 min at a distance r_{Rx} of 50 μm and for a frequency ω equal to 0. The curves of the delay Δ always show a monotonically decreasing trend as function of the frequency ω, more pronounced for higher values of the distance r_{Rx}.

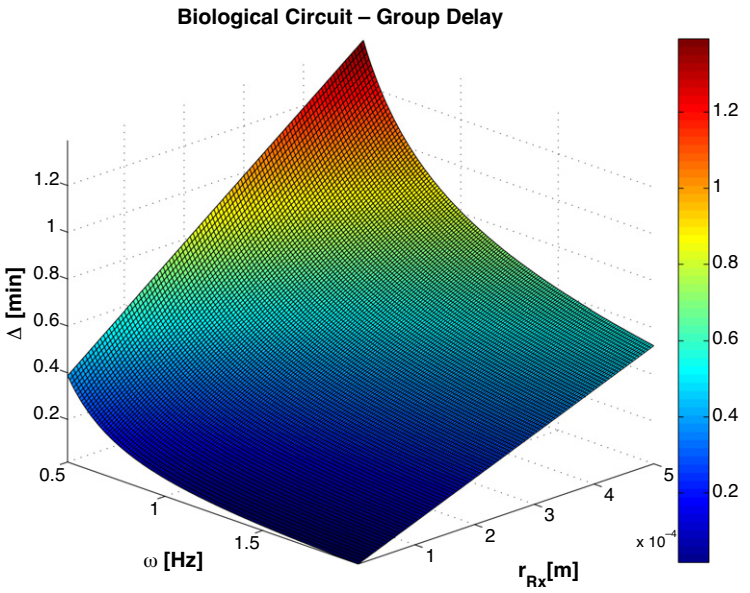

Fig. 5. Delay of the biological circuit for diffusion-based MC as function of the receiver distance from the transmitter r_{Rx} and the frequency ω.

5. Conclusion

In this paper, a systems-theoretic model is derived for a diffusion-based molecular communication system design based on biological circuits. Biological circuits are defined as genetic regulatory networks embedded in a biological cell, and they are envisioned to allow the future engineering of complete biological nanomachines. Some recent literature can be found on the analytical modeling of biological circuits, but with no specific mention to diffusion-based cell-to-cell communication through molecule exchange, for which only a biological description is provided in some specific works.

In our work, first, a biological circuit for diffusion-based molecular communication is described through a minimal subset of elements. Then, a mathematical model is detailed in terms of transfer functions, from which analytical expressions are derived for the attenuation and the delay experienced by an information signal through the biological circuits. Finally, we present some numerical results obtained by applying to the developed models some realistic biological parameters from the literature.

The work presented in this paper is a preliminary analysis of the application of communication engineering to the design of communication systems through biological circuits and their biochemical reactions within genetically-engineered cells. We believe this is the first step towards a potentially transformative interdisciplinary research area that could change the way we design devices and interact with the nature.

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