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# Genetically Engineered Bacteria-based BioTransceivers for Molecular Communication

Bige D. Unluturk, *Student Member, IEEE,* A. Ozan Bicen, *Student Member, IEEE,* Ian F. Akyildiz, *Fellow, IEEE*

***Abstract*—Molecular Communication (MC) is a nano-scale communication paradigm where the information is carried by molecular signals. To establish MC links, biological nanomachines can be utilized as transmitters and receivers. A bacterium can be programmed as a biotransceiver by modifying its genetic code to implement biological circuits. In this paper, genetically engineered bacteria-based biotransceivers are investigated for transmission of information between bacteria populations, where bacteria can generate and respond to the molecular signals. A biochemical model of biological circuits is presented, and both analog and digital signaling are studied. The challenges in connecting basic biological circuits to build these blocks are revealed. A biotransceiver architecture is introduced that combines sensing, transmitting, receiving and processing blocks. Furthermore, biological circuit design framework is proposed for transmission of signals with M-ary pulse amplitude modulation. The biological circuits designed for biotransceiver are elaborated via numerical results based on biochemical parameters of the genetically engineered bacteria. The provided results show that using biological circuits, transmitter and receiver functionalities can be implemented inside bacteria. Our work stands as a basis for future biotransceiver design for MC.**

***Index Terms*—Molecular communication, biological circuits, biotransceiver design, nanonetworks.**

## I. Introduction

BIOLOGICAL nanomachines and communication mechanisms, i.e, cells and intercell communication by molecules, already existing in nature can be exploited to build nanocommunication networks. This new communication concept based on the exchange of information carrying molecules is called Molecular Communication (MC) [1].

Most of the studies in the literature investigating MC mainly focus on the channel in which molecular concentration signals propagate and the channel effects on the communication performance. To realize MC networks, it is imperative to develop methods to generate these molecular signals for transmission and to interpret them after reception. This motivates us to study bacteria populations as nanomachines which inherently communicate with each other through molecules.

In this study, we aim to design a biotransceiver hardware as a communication device for MC nanonetworks by using genetically engineered bacteria populations. The proposed biotransceiver is capable of sensing, processing, transmitting, and receiving. To program the bacteria, molecular biologists modify the genetic code, i.e., the DNA of bacteria plasmid (Fig. 1), such that they can control the activity of the genes [2], [3]. Thus, the **gene expression**, i.e., the production of the proteins coded by those genes, can be controlled externally via changing the environmental conditions, i.e., the stimuli, which is called **gene regulation** [4]. A **biological circuit** provides signal transduction based on the modified gene sequences, the stimuli, and the proteins coded by the genes.

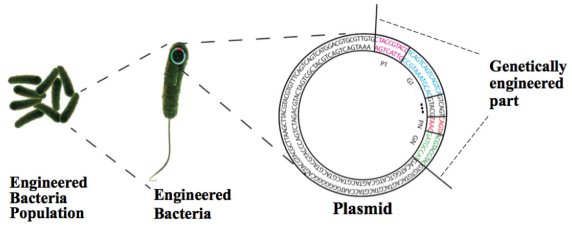


Fig. 1: Genetically engineered bacteria population produced by inserting genes to the plasmids of bacterial cells.

Biological circuits can be engineered using various techniques intervening in different stages of the gene expression such as transcriptional regulation [5], translational regulation [6] or spatial and temporal compartmentalization [7]. In our work, we consider the transcriptional regulation, i.e., controlling the synthesis of mRNA from DNA by inserting promoters before genes, which is one of the most studied regulation techniques [8]. More complex biological circuits can be built by combining all the above techniques.

Although there are several different biological circuit parts implemented in bacteria, e.g., logic gates [7], toggle switches [9], and concentration filters [10], there are several challenges in combining these parts for a more complex circuit as follows:

- **Orthogonality:** When a biological circuit containing multiple parts is implemented on the plasmid of a bacteria, all parts operate in the same cell environment simultaneously. If these parts include similar genes or proteins, there will be interference. Thus, to isolate each part from one another, a biological circuit design should consider using orthogonal elements, i.e., a molecule used in one biological circuit part should not interact with the rest of the biological circuit. This challenge prevents the recurrent use of the same circuit parts and modularity.
- **Timing and Delay:** Since each biological circuit part has a different delay, synchronizing the signals in the circuit is challenging. Besides, the gene expression pro-

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B. D. Unluturk, A. O. Bicen and I. F. Akyildiz are with the Broadband Wireless Networking Laboratory (BWN-Lab), School of Electrical and Computer Engineering, Georgia Institute of Technology, Atlanta, GA 30332 (e-mail: {bigedeniz, bozan, ian} @ece.gatech.edu). Phone: +1 404 894 6616, Fax: +1 404 894 7883.

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cess takes very long time, usually in the order of hours, which causes huge delays in biological circuits. Hence, concatenation of multiple biological circuit parts poses a severe limit on the information processing speed.

Considering these challenges, we present a concrete design for a complete nanomachine using MC for the first time in the literature. Furthermore, we lay down the principles of biological circuits from the view of electrical engineering for other scientists who may use them as a tool in their own nanomachine designs. Our work can be extended to form more complex biotransceivers or nanomachines by incorporating more detailed gene regulation techniques and stochastic properties of gene regulation.

The salient features of our work can be listed as:

1) *An overview of biochemical analysis of biological circuits:* We present a mathematical model for gene regulation by using reaction-rate equations (RRE) which include Hill functions. Then, we analyze biological circuits for analog and digital operations by approximating Hill functions as a linear and a step function, respectively. We present how biological circuit parts can be interconnected to form more complex circuits.
2) *Novel biotransceiver architecture:* Since the biological circuits have very limited capabilities, the complex architecture of electromagnetic transceivers cannot be adopted. Taking into account the peculiarities of biological circuits, a novel biotransceiver architecture with sensing, transmitting, processing and receiving blocks is presented.
3) *Biotransceiver design:* Biological circuits are designed for each block of the biotransceiver. The rates of different steps of gene regulation process are adjusted to equalize the delay of parallel paths and to ensure the timely delivery of signals to the next stages. The output signals of each part are designed such that the amplitudes of these signals stays in the defined range ensuring analog or digital operation of the consecutive circuit according to Hill function approximations.

The rest of the paper is organized as follows. In Sec. II, we review the principles of gene regulation, and we present a basic biological circuit. Section III analyzes a basic biological circuit unit and Section IV explains different gene-promoter configurations which will be used to connect the basic units together. In Sec. V, we propose a general transceiver architecture for genetically engineered bacteria based transceivers. Then, biological circuit designs for each block are provided in Section VI. Numerical results are presented in Section VII. Finally we conclude our study in Sec. VIII.

## II. Biochemical Model for Biological Circuits

To incorporate biological circuits into MC, the underlying biological phenomena of gene regulation in bacteria must be understood first. To regulate the expression of a gene, i.e., to control the production of the protein coded by that gene, an external stimuli is applied to the environment where the genetically engineered bacteria live. The external stimuli can be created by adding reactive molecules in the environment or by changing the environmental conditions such as light, pH, oxygen level [4]. According to the amount of the external stimuli, bacterial cell adjusts the production rate of the protein coded by the gene. In MC, the information can be encoded in the concentration, type, or arrival time of the molecules. Here, we assume that the information is encoded in the concentration of the molecules, and we investigate the gene regulation dynamics for the application of molecular concentration signals as stimuli to biological circuits.

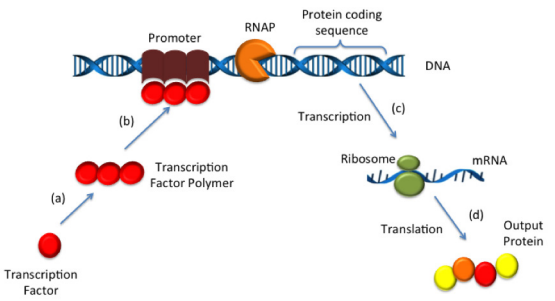


Fig. 2: Gene regulation processes. (a) The transcription factor (TF) forms a polymer. (b) TF binds to the promoter helping RNAP to separate DNA. (c) DNA is transcripted into mRNA. (d) Ribosomes read the mRNA and produce the protein.

Changes in bacterial gene expression are often mediated by the regulator sites on DNA called **promoter**. The promoter sites can bind to the molecules called **transcription factors** which control the expression level of the genes coded in DNA after the corresponding promoter site. The basic unit is composed of a gene and its corresponding promoter specially designed to bind and respond to a transcription factor, which acts as an **activator** enhancing the protein production or **repressor** inhibiting the protein production as illustrated in Fig. 2. The produced protein as the output of this basic unit might act as a transcription factor for another.

### A. Chemical Kinetic Model of Gene Regulation

The regulation of a gene constitutes of multiple steps, namely, polymerization, promoter binding, transcription, translation and natural decay illustrated in Fig. 2 [12]. In this subsection, each step is explained in detail and the governing chemical reaction for each step is provided.

1) *Polymerization*: Usually, the transcription factor added to the medium as the input signal cannot bind the promoter directly. Multiple molecules of the transcription factor bind to each other to take an active form which is suitable for binding. The combination process of $n$ molecules of transcription factor denoted with $X$ is called *polymerization*. When $n$ increases, the cooperativity of that transcription factor increases and the system becomes more robust to the abrupt changes of $X$. The polymerization reaction can be characterized as

$$nX \underset{k_{p0}}{\overset{k_p}{\rightleftharpoons}} X_n. \tag{1}$$

where $k_p$ is the rate of forward polymerization reaction, and $k_{p0}$ is the rate of the reverse reaction.

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2) *Transcription factor-promoter binding*: The polymer of $X$ binds to the promoter $P$ on DNA next to its corresponding gene forming a complex $C$. After this binding, the promoter is activated and RNA polymerase denoted by $RNAP$ sticks to the promoter. The binding reaction is

$$X_n + P \underset{k_{b0}}{\overset{k_b}{\rightleftharpoons}} C, \tag{2}$$

where $k_b$ is the rate of forward binding reaction, and $k_{b0}$ is the rate of the reverse reaction.

3) *Transcription*: If the transcription factor is an activator then the chemical affinity of the promoter and $RNAP$ increases which means that $RNAP$ binds to the promoter more easily resulting in a boost in $mRNA$ production rate. If the transcription factor is a repressor, then the chemical affinity decreases, so the $mRNA$ production rate. This step is called *transcription* since the information about the protein coded on DNA is copied to $mRNA$. The $mRNA$ production from the unactivated promoter, i.e., $P$, corresponding to the basal level of protein production is described as

$$P + RNAP \xrightarrow{\alpha_0} P + RNAP + mRNA, \tag{3}$$

where $\alpha_0$ is the basal $mRNA$ production rate. The $mRNA$ production from the activated promoter, i.e., C, is described as

$$C + RNAP \xrightarrow{\alpha_1} C + RNAP + mRNA, \tag{4}$$

where $\alpha_1$ is the regulated $mRNA$ production rate.

4) *Translation*: Ribosomes in the cell bind to the $mRNA$ produced in the transcription step and read the information on $mRNA$ about the number, the order, and the type of the aminoacids forming the output protein $Y$. Starting from one end of the $mRNA$, ribosomes composed of rRNAs form a chain of aminoacids which will fold and take its final form as the output protein. As the sequence of bases in $mRNA$ are interpreted as the chain of aminoacids, this step is called the *translation* which is described by

$$mRNA + rRNA \xrightarrow{k_t} mRNA + rRNA + Y, \tag{5}$$

where the transcription rate is given as $k_t$.

5) *Natural Decay*: All the proteins are degraded by the enzymes in cell after they complete their lifetime. This *natural decay* is described by

$$mRNA \xrightarrow{\gamma_M} \emptyset, \tag{6}$$

$$Y \xrightarrow{\gamma_Y} \emptyset, \tag{7}$$

$$X \xrightarrow{\gamma_X} \emptyset, \tag{8}$$

$$X_n \xrightarrow{\gamma_{X_n}} \emptyset, \tag{9}$$

where decay occurs for $i^{th}$ species with rate $\gamma_i$.

### B. Reaction-Rate Equation Model of Gene Regulation

Chemical processes in gene regulation consist of discrete and stochastic components and exhibit a very noisy behavior. When the number of molecules of each specie is large, the change in concentrations can be accurately calculated by the law of mass action using RRE [11]. Since in this work we are considering a population of bacteria containing thousands of bacterium, the number of molecules can be approximated by a continuous molecular concentration and the chemical processes can be approximated by an RRE model, which gives the mean of the stochastic molecular signals. This approach is widely adopted in MC literature based on genetically engineered nanomachines and it is experimentally verified in [4], [20]. The use of RRE model simplifies the analysis, where the effects of the number of cells and the spatial distribution of cells are implicitly captured in the reaction rates. The incorporation of these effects into the model as additional parameters is beyond the scope of this paper.

In our model, we consider that the differences between each individual bacteria are assumed to be averaged out such that we observe the mean response of the population [11]. Furthermore, the diffusion of the molecules into bacterium and out of bacterium are not taken into consideration, since the diffusion takes places very rapidly compared to translation and transcription processes [14]. Besides, it is considered that the environment contains plenty of oxygen and nutrients such that the bacterial population density is maintained, and the bacteria have enough energy to express the regulated genes. Also, it is assumed that the biofilms formed by bacteria are weak and they do not limit the diffusion of molecules.

The derivation of RRE from the chemical reactions using the law of mass action is explained in the Appendix. Using this method, we converted the chemical reactions of gene regulation (1)-(6) into the reaction-rate equations (10)-(15). Accordingly the rate of change of the input signal, i.e., the transcription factor, $X$, is given by

$$\frac{dX}{dt} = k_{p0} X_n - n k_p X^n - \gamma_X X, \tag{10}$$

which is obtained by reactions (1) and (6).

The rate of change of the transcription factor polymer is

$$\frac{d(X_n)}{dt} = k_p X^n + k_{b0} C - k_b X_n P - \gamma_{X_n} X_n, \tag{11}$$

which is obtained based on reactions (1), (2) and (6).

The rate of change of the free promoter $P$ is

$$\frac{dP}{dt} = k_{b0} C - k_b X_n P, \tag{12}$$

whereas the rate of change of the bound promoter $C$ is

$$\frac{dC}{dt} = k_b X_n P - k_{b0} C, \tag{13}$$

which are obtained by reaction (2).

The rate of change of $mRNA$ is obtained from reactions (3) and (4) as

$$\frac{d(mRNA)}{dt} = \alpha_0 P + \alpha_1 C - \gamma_M mRNA. \tag{14}$$

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Finally, the rate of change of the output protein $Y$ found from reaction (5) is

$$\frac{dY}{dt} = k_t(mRNA)(rRNA) - \gamma_Y Y. \quad (15)$$

The output protein concentration, $Y$, can be determined by solving the ordinary differential equation (ODE) set (10-15). However when there are multiple transcription factor-gene pairs, multiple coupled sets of ODE's should be considered which is difficult to solve analytically. In Section III, methods for simplifying this ODE set are introduced.

## III. Analysis of Regulated Gene Expression

RRE models capture the dynamical nature of biological processes. However, these models are not easy to analyze when the complexity of the biological circuit increases [17]. Here, we simplify (10)-(15) by using three approaches, namely, elimination of irrelevant species, time-scale differences, and Michaelis-Menten approximation [4].

The model in (10)-(15) captures also the concentration of intermediate species like transcription factor-promoter complexes and RNAP which are not the essential points of interests. Thus, it is important to simplify the model by removing these irrelevant species [4]. To do so, time scale differences between the fast reaction of promoter binding (2) and the slow reactions of translation (5) and transcription (3,4) are exploited. We consider that the fast reactions are in steady-state. Thus, we assume that there is not a significant change in the concentrations of $X$ and $X_n$. Then, we combine the equations (10)-(13) as

$$\frac{dC}{dt} = k_b P X^n - k_{b0} C, \quad (16a)$$

$$\frac{dP}{dt} = -\frac{dC}{dt}. \quad (16b)$$

Due to the conservation of mass principle, the total amount of free or bound promoter sites is constant, i.e., $P + C = P_T$. Setting the equations for C and P at quasi steady-state, i.e., $dC/dt \approx 0$, we obtain

$$C = P_T \frac{X^n}{\theta_X^n + X^n}, \quad (17a)$$

$$P = P_T \frac{\theta_X^n}{\theta_X^n + X^n}, \quad (17b)$$

with $\theta_X = (k_b/k_{b0})^n$. The amount of mRNA molecules depends both on the concentration of promoter sites bound to an activator or repressor, and on the amount of free promoters. Since the production of mRNA depends linearly on $P$ and $C$, mRNA decays at a constant rate $\gamma_M$.

If the transcription factor is an activator, the contribution of $C$ to $mRNA$ production is much larger than that of $P$, which is represented by $\alpha_1 >> \alpha_0$. Substituting the quasi-steady state expressions for $C$ and $P$ in (17) into (14) yields:

$$\frac{d(mRNA)}{dt} = \kappa_0 + \kappa_1 \frac{X^n}{\theta_X^n + X^n} - \gamma_M(mRNA), \quad (18)$$

where $\kappa_0 = \alpha_0 P_T$ and $\kappa_1 = (\alpha_1 - \alpha_0)P_T$.

If the transcription factor is a repressor, the contribution of $C$ to $mRNA$ production is much smaller than that of $P$, i.e., $\alpha_1 << \alpha_0$. In this case, (14) becomes

$$\frac{d(mRNA)}{dt} = \kappa_0 + \kappa_1 \frac{\theta^n}{\theta_X^n + X^n} - \gamma_M(mRNA), \quad (19)$$

where $\kappa_0 = \alpha_1 P_T$ and $\kappa_1 = (\alpha_0 - \alpha_1)P_T$. In both cases, the parameter $\kappa_0$, which is much less than $\kappa_1$, denotes a residual or basal activity considered to be 0.

The next step is translation, shown with the following equation derived from (15) in the abundance of rRNA

$$\frac{dY}{dt} = k_t(mRNA) - \gamma_Y Y. \quad (20)$$

So the simplified set of equations is

$$\begin{aligned} \frac{d(mRNA)}{dt} &= \kappa_1 f^{\pm}(\theta_X, X, n) - \gamma_M(mRNA), \\ \frac{dY}{dt} &= k_t(mRNA) - \gamma_Y Y, \end{aligned} \quad (21)$$

where $\kappa_1 = (\alpha_1 - \alpha_0)P_T$. The expression $f^{\pm}(\theta_X, X, n)$ is called Hill function. For the activator case, Hill function becomes $f^+(\theta_X, X, n) = X^n/(\theta_X^n + X^n)$ while for the repressor case, $f^-(\theta_X, X, n) = \theta_X^n/(\theta_X^n + X^n)$. For gene regulatory networks, the exponent $n$ is considered to be large $(n >= 2)$ according to the experimental data [18]. For large $n$, the parameter $\theta_X$ has therefore a special meaning: it is a threshold value below which there is practically no activity and above which activity is (almost) maximum. In the limit as $n$ tends to infinity, the Hill function becomes a step function.

Similar to electrical circuits, we can define analog and digital operation for biological circuits too [22]. In this study, we differentiate these two operation modes according to the operation region of Hill function.

### A. Analog Interpretation of Biological Circuits

For analog operation, we consider that the input concentration falls down to the interval where the Hill function acts almost as a linear function [21]. Assuming an oscillatory input $X = \theta_X + X_0 cos(2\pi f_0 t)$, we approximate the Hill function as $f^+(\theta_X, X, n) = 1/2 + \frac{n}{4\theta_X}(X - \theta_X)$ calculated by Taylor's expansion for $X_0 \ll \theta_X$. By applying Fourier transform to (21), the transfer function of an activator can be found as

$$H(j\omega) = \frac{\kappa_1 k_t n}{4\theta_X (j\omega + \gamma_M)(j\omega + \gamma_Y)}. \quad (22)$$

The transfer function of an activator expressed in (22) shows low-pass characteristics. Since mRNA decays much faster than the protein $(\gamma_M \gg \gamma_Y)$, for frequencies higher than $\gamma_Y$, $H(j\omega)$ decreases rapidly [23]. The analog operation of a basic biological circuit unit illustrated in Fig. 2 can be seen in Fig. 3 where an input concentration signal with multiple frequencies is applied. We observe that the higher frequencies are suppressed by the biological circuit due to the low-pass characteristics of the biological circuit.

Since the repressor shows the same frequency domain characteristics, we conclude that the basic biological circuit units can follow input signals up to critical frequency, $f_c = \gamma_Y/2\pi$,

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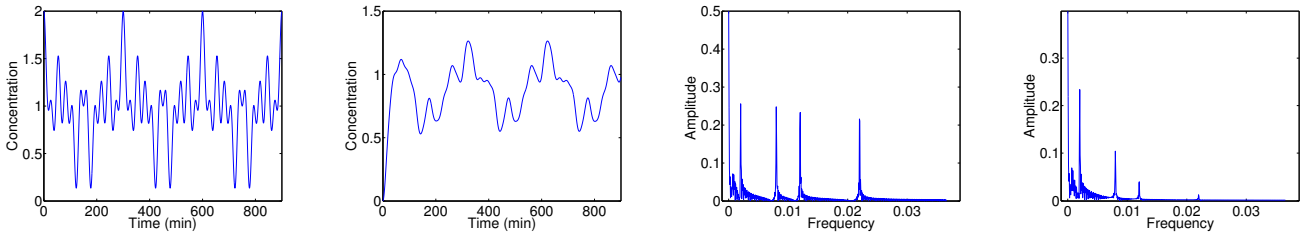


(a) (b) (c) (d)

Fig. 3: Analog operation of the basic biological circuit unit. (a) Applied input signal, i.e., the concentration of the transcription factor X is presented which is composed of the mixing of three different frequencies 0.005, 0.007 and 0.01 Hz. (b) The output signal, i.e., the concentration of the protein Y is presented, which is smoother than the input signal. (c) The frequency spectrum of the input signal. (d) The frequency spectrum of the output signal whose higher frequencies are suppressed.

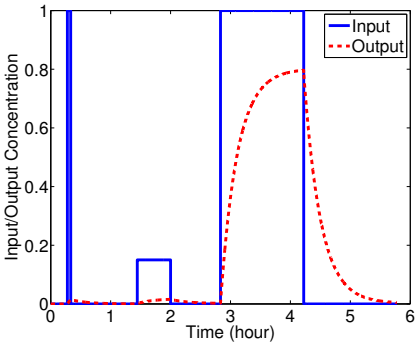


Fig. 4: Operation of the basic biological circuit unit. The output is produced when a high enough input concentration is applied during a long enough time.

which settles an upper limit for the operation frequency of biological circuits. For the linear approximation of Hill function, the biological activator circuit resembles a transistor which operates as an amplifier biased with a DC voltage [23].

### B. Digital Interpretation of Biological Circuits

In electrical circuits, transistors are also used as digital switches. To obtain a switch from biological circuits, it is considered that for the high cooperativity $n$, the Hill function becomes a step function [2]. For an activator, it is assumed that $mRNA$ production is zero below the threshold $\theta_X$, and mRNA production is maximum above the threshold $\theta_X$. It is expressed as $f(\theta_X, X, n) = I(X > \theta_X)$ for an activator and $f(\theta_X, X, n) = I(X < \theta_X)$ for a repressor, where $I$ denotes the unit step function. The step function approximation of the Hill function may not fully describe the transient response of a biological circuit but it describes the state information of the circuit effectively [16]. For digital operation, the input is defined as the molecular concentration signal alternating between the levels '0' and '$N_c$' $\mu mol/V$ representing '0' and '1' bit according to the message. An input concentration signal $x(t)$ to an activator behaving like a switch is assumed to be a pulse of amplitude $N_c$ and width $T$, i.e., $x(t) = N_c$ for $t < T$ and $x(t) = 0$ for $t > T$. The output protein concentration $y(t)$ can be calculated as $y(t) = Y_{st}(1 - e^{-\gamma_y t})$ by solving the differential equation system in (21) where $Y_{st} = \kappa_1 k_t/(\gamma_y \gamma_M)$ and the delay is $\tau_d = ln(2)/\gamma_y$.

For the given input, the maximum value of the output depends on the duration of the input, $T$. When the input pulse finishes, the protein production stops and the output concentration starts to decrease due to natural decay. Thus, the maximum value that $y(t)$ can reach is its concentration at $t = T$ which is $y_{max} = Y_{st}(1 - e^{-\gamma_Y T})$. Then, the decaying output concentration after $t > T$ is expressed as $y(t) = Y_{st}(1 - e^{-\gamma_Y T})e^{(-\gamma_Y (t-T))}$.

The output concentration $y(t)$ decays exponentially. Even though it will take long time to totally vanish because of exponential decay, $y(t)$ will be significantly reduced after $\tau_y = 1/\gamma_Y$. The digital operation of the basic biological circuit unit can be seen in Fig. 4 where in order to obtain digital pulses as outputs, the input molecule concentration should be long enough and should have high enough amplitude.

Up to now, we considered a basic biological circuit unit comprising only a single promoter and a single gene. By combining these units we can build more complex circuits.

## IV. Interconnecting Basic Biological Circuit Units

To build functional circuits we need to connect the basic units together using three different configurations, namely, single input single output (SISO), multiple input single output(MISO), single input multiple output (SIMO) [2]. Using these configurations, an input signal can be fed to different paths and signals coming from different paths can be merged.

### A. Single Input Single Output Configuration

In SISO configuration, there is a single input transcription factor controlling the concentration of a single output protein. The activating characteristic is shown by an arrow in Fig. 5 (a) while the repressing characteristic is shown by a flat-end arrow in Fig. 5 (b). In both figures, squares represent promoters, rectangles represent genes, where A is the input transcription factor and B is the output protein produced from that gene.

### B. Multiple Input Single Output Configuration

In this configuration illustrated in Fig. 6, a single gene is controlled by two activator transcription factors A and C

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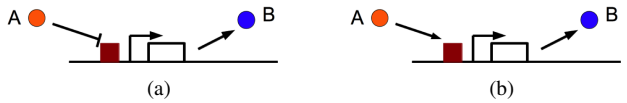


Fig. 5: SISO Configuration. (a) The transcription factor A binds to the promoter activating the production of the molecule B. (b) The transcription factor A binds to the promoter and stops the production of B.

which may activate the gene either collaboratively or additively. If they work collaboratively, both of the transcription factors need to be present to activate the gene and the mRNA production rate $\kappa_1 f(\theta_X, X, n)$ in (21) is replaced by $\kappa_{AC} f(\theta_A, A, n) f(\theta_C, C, n)$. If they work additively, either of the transcription factors may activate the gene independently and the total mRNA production rate is the sum of the contributions of the two transcription factors. The total mRNA production rate in this case is expressed as $\kappa_A f(\theta_A, A, n) + \kappa_C f(\theta_C, C, n)$. When one or both of the transcription factors

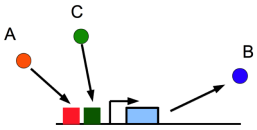


Fig. 6: Multiple Input Single Output Configuration

are repressors, the functions $f(\theta_A, A, n)$ and $f(\theta_C, C, n)$ are replaced by the repressor Hill functions. This configuration serves for merging two signals coming from different sources.

### C. Single Input Multiple Output Configuration

In this configuration illustrated in Fig. 7, a promoter is controlling two different genes producing proteins B and C simultaneously with the same rate. By using multiple outputs, two molecular concentrations representing the same signal are obtained, where one may be used for monitoring while the other is propagated to the latter parts of the circuit.

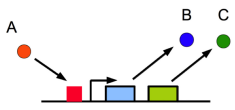


Fig. 7: SIMO Configuration. The transcription factor A binds to the promoter producing the proteins B and C proportionally.

## V. Biotransceiver Architecture

A transceiver for MC should be capable of sensing the environment, receiving signals from other MC devices, processing the received and sensed information and finally transmitting the processed information as molecular signals. To this end, we propose the architecture illustrated in Fig. 8.

In our transceiver architecture, we adopt a baseband pulse transmission scheme where the molecular signals are subject to channel effects and noise. For the sake of simplicity we

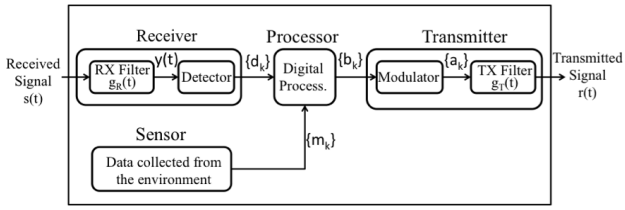


Fig. 8: Transceiver architecture for MC illustrating the connections between its functional blocks.

adopt a coded modulation scheme. The transmitter modulates the bit sequence $\{b_k\}$ received from the processor block and generates the symbol sequence $\{a_k\}$. Then, the transmit filter $g_T(t)$ generates a pulse according to the symbol $\{a_k\}$ and produces the transmitted signal $r(t)$. All types of MC channels such as diffusion-based, flow-based or walkway-based distort the transmitted signal $r(t)$ and add noise. To reconstruct the original signal from the distorted signal $s(t)$, a receiver filter followed by a detector is used which regenerates the intended bit sequence $\{d_k\}$ from $y(t)$. The sensor block of the architecture collects data from the environment and generates a bit sequence $\{m_k\}$. Then, the processor block merges the information generated in the sensor block $\{m_k\}$ with the information coming from the other nanomachines $\{d_k\}$ and produce an output bit sequence $\{b_k\}$ to be transmitted.

The link between two different bacteria populations can be constructed only if the transmitted signal of one population is using the same type of molecule that the other bacteria population is intended to receive. If all biotransceivers transmit different types of molecules, the identity of the biotransceiver will be inferred from the molecule type which solves the addressing problem and also it will avoid self-interference. However, this will require the production of different types of biotranceivers which is not that easy from the biological point of view. Furthermore, scalability problems may arise when creating a nanonetwork with a large number of biotransceivers. Otherwise, if all biotransceivers transmit the same type of molecule, the production of biotransceivers will be relatively easy despite the fact that it will cause inter-user interference, addressing and medium access control problems. In our work, we focus on the physical layer, these challenges about the link layer will be addressed in future research.

## VI. Biological Circuit Design for a Biotransceiver

Exploiting the mathematical abstraction provided in Section II and III, and the interconnections defined in Section IV, we design representative biological circuits for each block of the biotransceiver architecture introduced in Section V for baseband pulse amplitude modulation.

### A. Transmitter

The function of the transmitter is the generation of molecular concentration signals $r(t)$ according to the data to be transmitted represented by the bit sequence $\{b_k\}$. It consists of a modulator which creates symbols $\{a_k\}$ from the bit sequence

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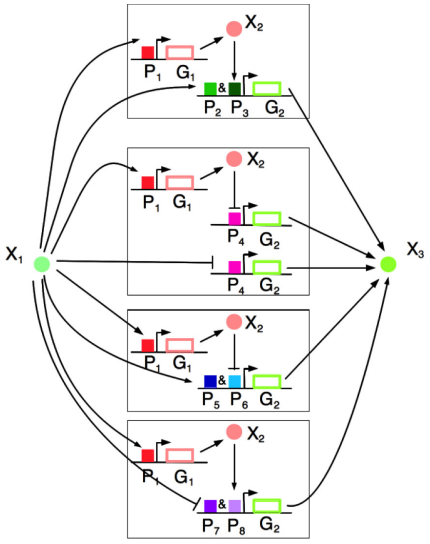


Fig. 9: M-ary modulator biological circuit. The input protein $X_1$ represents digital bits which are translated into M concentration levels in the output protein $X_3$.

$\{b_k\}$ and a transmission filter $g_T(t)$. The transmitted signal at the output of the transmitter can be expressed as

$$s(t) = \sum_{k=0}^{\infty} a_k g_T(t - kT), \tag{23}$$

where $T$ is the bit duration.

*1) Modulator:* In MC literature, there are numerous modulation techniques such as concentration shift keying, frequency shift keying, pulse position modulation, and molecular shift keying. Since MC channels are often very slow [13], they do not support utilization of high frequency carriers. The most suitable modulations techniques are On-Off Keying (OOK) and M-ary amplitude modulation, where the information is encoded on the amplitude of baseband MC signal.

For this design, we adopt M-ary amplitude modulation. Converting the consecutive bits into different amplitude levels representing the symbols, requires many parallel biological circuits. Here we consider a M-ary modulation scheme with M=4. Since a concentration cannot be negative, all symbols are positive and equally distant to each other assigned as follows:

$$\{a_k\} = \begin{cases} 4A_0, & \text{if } (b_k, b_{k-1}) = (1,0) \\ 3A_0, & \text{if } (b_k, b_{k-1}) = (1,1) \\ 2A_0, & \text{if } (b_k, b_{k-1}) = (0,1) \\ A_0, & \text{if } (b_k, b_{k-1}) = (0,0) \end{cases} \tag{24}$$

where the Gray coding is used to minimize the error.

To implement this M-ary modulator, we propose the circuit in Fig. 9 where the concentration of the protein $X_1$ is the input of the modulator representing bit sequence $\{b_k\}$ and the concentration of the protein $X_3$ is the output representing symbol sequence $\{a_k\}$. Here we assume that the circuit in Fig. 9 operates as a digital circuit as described in Section III-B.

The basic unit composed of $P_1$ and $G_1$ pair serves as a unit delay element. When $k = i$, $X_2$ represents the previous input $b_{i-1}$ where $X_1$ represents the input $b_i$. In all the four

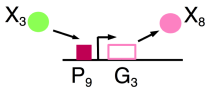


Fig. 10: Transmit filter biological circuit operates in analog mode and shapes the modulator output $X_3$ into $X_8$.

branches depicted in Fig. 9, the previous input is compared with the current input and each branch is activated only for one of the possible input sequences $\{(1,1),(0,0),(1,0),(0,1)\}$, respectively from the top to the bottom, respectively.

When activated, each branch produces the same output molecule but with different rates $\kappa_1^i$ which provides different amplitude levels as symbols. In ideal case, $\{a_k\}$ is composed of impulses. However, in the practical biological circuits, according to Section III-B, $\{a_k\}$ corresponds to consecutive pulses $p^i(t)$ of the form

$$p^i(t) = \begin{cases} \frac{\kappa_1^i k_t}{\gamma_{X_3}\gamma_M}(1 - e^{-\tau t}), & \text{if } t < T_b \\ \frac{\kappa_1^i k_t}{\gamma_{X_3}\gamma_M} e^{-\tau T_b} e^{-(t-T_b)}, & \text{if } t \geq T_b \end{cases} \tag{25}$$

where $i \in \{P_2\&P_3, P_4, P_5\&P_6, P_7\&P_8\}$ and the continuous signal $a(t)$ is expressed as $a(t) = \sum_{k=-\infty}^{\infty} p^i(t - kT)$. Thus, we can map $\{a_k\}$ to $\kappa_1^i k_t/(\gamma_{X_3}\gamma_M)$. Since $k_t, \gamma_{X_3}, \gamma_M$ are the same for all branches, we can find $\kappa_1^i$ as follows $\kappa_1^{P_2\&P_3} = 3\kappa_{1o}, \kappa_1^{P_4} = \kappa_{1o}, \kappa_1^{P_5\&P_6} = 4\kappa_{1o}, \kappa_1^{P_7\&P_8} = 2\kappa_{1o}$.

Here, as the color coding in Fig. 9 indicates, each branch is using different promoters to establish the orthogonality. Since the delay of a basic biological circuits unit depends only on the decay rate of the output protein as specified in Section III-B, even though the four branches include different promoters, they have the same delay $t_d = ln(2)/\gamma_{X_3}$. So for all four branches, the total delay is $t_d^{mod} = ln(2)/\gamma_{X_2} + ln(2)/\gamma_{X_3}$.

*2) Transmit Filter:* The symbols $\{a_k\}$ are fed to the transmit filter $g_T(t)$ which generates a rectangular pulse for each symbol ideally. In this study, we consider a simple low-pass transmit filter which corresponds to the activator configuration depicted in Fig. 10 used in the analog operation mode described in Section III-A. Using the equation (22), the transfer function of $\hat{g}_T(t)$ can be expressed as

$$\hat{G}_T(j\omega) = \frac{\kappa_1^{P_9} k_t n}{4\theta_{X_3}(j\omega + \gamma_M)(j\omega + \gamma_{X_2})}, \tag{26}$$

where the output of the transmit filter, i.e., the output of the transmitter block is $y(t) = a(t) * \hat{g}_T(t)$. Note that since the modulator block is not ideal, the actual transmit filter $g_T(t)$ is expressed as $g_T(t) = p(t) * \hat{g}_T(t)$. To guarantee that the transmit filter operates always in the analog mode, the concentration of input $X_3$, i.e, the modulated signal $a(t)$ must be in the linear range of the Hill function, thus $\theta_{X_2} - \frac{2\theta_{X_2}}{n} < a(t) < \theta_{X_2} + \frac{2\theta_{X_2}}{n}$ should hold.

## B. Sensor

Biological circuits can sense a variety of environmental conditions such as light, temperature, and presence of food or poison. When there is a change in the environmental

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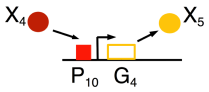


Fig. 11: Sensory biological circuit reports the changes in $X_4$ to the processor by producing the protein $X_5$.

conditions, the rate that the promoters activate the genes changes resulting in a change in the concentration of proteins.

A basic sensory block can be composed of a single activator circuit operating in digital mode. When the stimuli is above a threshold, the output protein concentration will go to high level while the stimuli is below the threshold, the output concentration will drop to 0. By this sensor design, an on-off sensor or a hypothesis testing sensor can be build. An example circuit is shown in Fig. 11. The sensor design can be extended to more sensitive sensors with higher precision, by combining several of these 1 bit sensors.

The bit sequence $m_k$ represents the output of the sensor block and corresponds to the concentration of $X_5$. The concentration of $X_4$ is denoted by $d(t)$ and $m_k$ is

$$m_k = \begin{cases} C_0, & \text{if } d(t) > \theta_{X_4} \\ 0, & \text{if } d(t) < \theta_{X_4} \end{cases} . \quad (27)$$

### C. Processor

The processor considered in this study is composed of logic gates and memory elements demonstrated to be implementable by synthetic biology studies [24].

*1) Logic Gates:* Recently biologists have implemented all the logic gates in bacteria. Although complex logic gates such as NAND, NOR can also be constructed by biological circuits, only NOT, AND and OR gates are described in this paper.

**NOT:** A NOT gate is a repressor circuit which produces molecule B when it is not stimulated. When the transcription factor A is present, the promoter is repressed so the gene becomes deactivated and molecule B production stops.

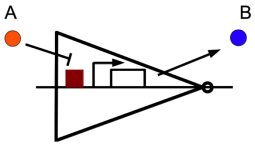


Fig. 12: NOT gate composed of a repressing biological circuit where B is the logical inverse of A.

**AND:** By the means of the MISO configuration described in Section IV-B, an AND gate can be constructed using only a single basic biological circuit unit. The gene is activated only when both of the transcription factors are present.

**OR:** The OR gate constitutes of two basic biological circuit units that produce the same output molecule. When one or both of the inputs are present, the output protein is produced.

Since there is no isolation between biological circuit units, designing a circuit with multiple logic gates requires choosing orthogonal, i.e., noninteracting promoters and genes for each gate. This orthogonality problem prevents the multiple use of

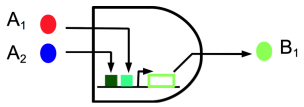


Fig. 13: AND gate including two promoters which activate the gene collaboratively. $B_1$ is the logical 'AND' of $A_1$ and $A_2$.

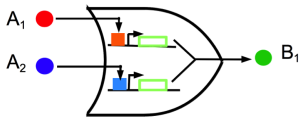


Fig. 14: OR gate composed of two parallel circuits which activate the same gene independently. $B_1$ is the logical 'OR' of $A_1$ and $A_2$.

the same gate. On the contrary, in electrical circuits, it is favorable to use the same gate for the whole design due to transistor fabrication simplicity.

*2) Memory:* Memory in biological circuits is achieved by a toggle switch which includes two promoters which are effected by two inducers [9]. One of the two inducers sets the switch into one of the stable states, and the other sets it to the other stable state. Then, the toggle switch holds the state even the inducers are drawn back.

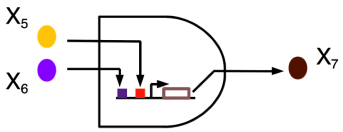


Fig. 15: Processor circuit having two promoters binding to inputs $X_5$ and $X_6$ which are both required to activate the gene producing $X_7$. This block acts as an AND gate.

In this paper, an AND gate, combining the sensor data with the received data is used as the processor as shown in Fig. 15. For example, the sensor measures the food level. When the food level is above the threshold, the sensor gives a high output which allow the processor to transfer the received data to the transmitter block. Thus, the bacterium relays a message only if there is food.

### D. Receiver

This block identifies the incoming molecular signal, measure its amplitude and convert the information encoded in the amplitude of the molecular signal to digital bits. For amplitude-based modulation techniques, concentration band detector circuits [12] can be used to identify different amplitude levels. The received signal $s(t)$ is first passed through a receive filter $g_R(t)$ and $y(t)$ is obtained. Then, the detector determines the intended bits by thresholding $y(t)$.

*1) Receive Filter:* In classical communication the receive filter is used to shape the pulse for optimal detection in the presence of channel noise. The optimal filter is the matched filter when the noise in the channel is additive white noise.

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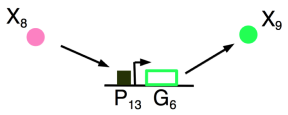


Fig. 16: Receive filter biological circuit operating in analog mode which filters the received signal $X_8$ and outputs $X_9$.

However, with biological circuits it is not easy to create the matched filter. Thus, a low-pass filter illustrated in Fig. 16 is used as the receive filter to lower complexity of the receiver yet reducing noise. Using the equation (22), the transfer function of $g_R(t)$ can be expressed as

$$G_R(j\omega) = \frac{\kappa_1^{P_{13}} k_t n}{4\theta_{X_8}(j\omega + \gamma_M)(j\omega + \gamma_{X_9})}, \tag{28}$$

where the output of the receive filter is $y(t) = s(t) * g_R(t)$. Note that to avoid self-interference, the molecules used for $r(t)$ and $s(t)$ are different.

*2) Detector:* In classical communication the output of the receive filter is sampled and applied to a threshold detector. In this study, since sampling with biological circuits is not feasible, we will directly apply thresholding to the filtered received signal $y(t)$. In order to do so, four parallel branches of biological circuits are designed as seen in Fig. 17 using activator circuits in digital mode where the promoter of each branch has a different activation threshold. If $y(t)$ is above that threshold that branch is activated and produce the output molecule representing the bit sequences $\{m_k\}$.

Remembering (24) and assuming each symbol is equally likely, detection thresholds of each branch expressed as $\theta_{P_{13}} = 3.5A_0, \theta_{P_{14}} = 2.5A_0, \theta_{P_{15}} = 1.5A_0, \theta_{P_{16}} = 0.5A_0$.

The proteins $X_9, X_{10}, X_{11}, X_{12}$ are produced for the following intended bit sequences

$$\begin{aligned} X_9 &\rightarrow \{(1,0)\} \\ X_{10} &\rightarrow \{(1,0),(1,1)\} \\ X_{11} &\rightarrow \{(1,0),(1,1),(0,1)\} \\ X_{12} &\rightarrow \{(1,0),(1,1),(0,1),(0,0)\} \end{aligned} \tag{29}$$

from which we can deduce how to generate $\{m_k\}$ according to the these proteins. For the first bit of $\{m_k\}$, it is enough to check $X_9$ and $X_{11}$. If they are both present than they activate the gene $G_{11}$ which produces a pulse representing the first 1 bit of the sequence. Then, for the second bit, it is enough to check $X_{10}$. If it is present, then the gene $G_{12}$ is activated. To adjust the timing of the second bit, we include an intermediary stage after $G_{12}$ such that it is expressed as the second bit after the expression of the first bit.

## VII. Numerical Results

In this section, we investigate the operation of the bio-transceiver proposed in Section VI. The analytical expressions in time domain given in Section III and VI are evaluated.

In this study, E. Coli is assumed to be the host bacteria for the biological circuits since the genetic manipulation of E. Coli is well-studied in synthetic biology literature. The evaluation

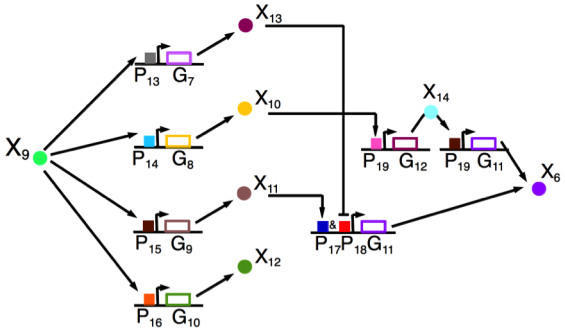


Fig. 17: Detector circuit compares the filtered received signal $X_9$ with the activation thresholds of the four branches' promoters on the left-hand side to detect which M-ary amplitude level was transmitted. Then, the right-hand side generates the bit sequence according to the detector concentrations.

parameters are taken from the literature and scaled up or down in the same order. The promoter activity is $\kappa_1 = 10$ [25], translation rate is $k_t = 150\ \mathrm{h}^{-1}$ [20], protein decay rate is $\gamma_Y = 4.15\ \mathrm{h}^{-1}$ [26], mRNA decay rate $\gamma_M = 10.05\ \mathrm{h}^{-1}$ [26], and bit duration $T_b = 30$ min. For simplicity we assumed that decay rates and translation rates for every promoter-gene pair are equal which in turn equalize the delay of each basic unit. Also, in the rest of this section, the molecular concentration signals presented are all normalized such that the maximum concentration of a pulse representing bit 1 is 1 $\mu$M.

Using libraries of genetic parts such as BioBricks [19], promoter-gene pairs which have parameters in the order of the designed values can be found. Then, to tune the parameters, three different techniques may be used [3]. The first one is the iterative rational design where the performance is experimentally evaluated at each step and the system design is modified until a specific performance requirement is met. The second technique is creating variants of the same circuits with different elements such as different promoters and testing all the variants until a suitable one is found. The third technique is the directed evolution which is based on mutation and selection. By using these techniques, the genetic circuit components used in the abstract circuit design can be mapped to actual promoter-gene pairs.

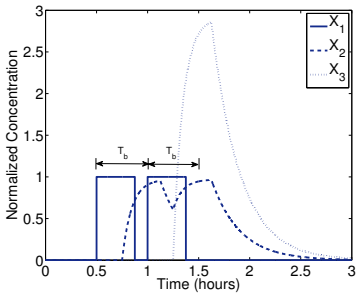


Fig. 18: Concentration of the input bits $X_1$, intermediary signal $X_2$, and the output M-ary modulated signal.

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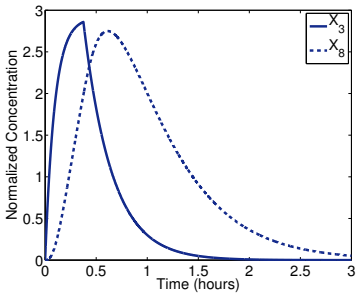


Fig. 19: Concentration of the input $X_3$ and the output $X_8$ of the transmitter filter which shapes the transmitted signal.

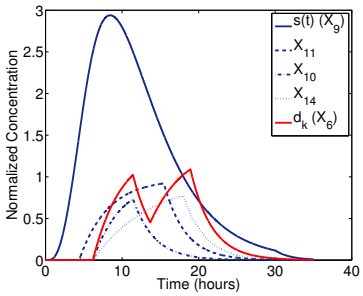


Fig. 21: The filtered signal s(t) and the signal representing the generated digital bits $d_k$ by the detector.

### A. Transmitter

Let us consider that there are two biotransceivers A and B. Assume that the processor of A generates a bit sequence $b_k$ of two consecutive bits with perfectly rectangular digital pulses that A encodes and sends to B. Assuming that the bit duration is $T_b$ and the line code is return-to-zero, the input $X_1$ and the output $X_3$ of the modulator described in Fig. 9 are considered for two consecutive bits '11'. In Fig. 18, it is observed that the symbol '3' is generated by the modulator according to (24). Note that the concentration signal representing the symbol peaks after the second bit finishes which indicates the delay which is approximately equal to the bit duration of 30 min.

After the symbols are generated the transmitter filter shapes the pulse as shown in Fig. 19. Here, $X_3$, is the input signal representing the symbol level and $X_8$ is the output of the transmitter, i.e., the signal put into the channel $r(t)$.

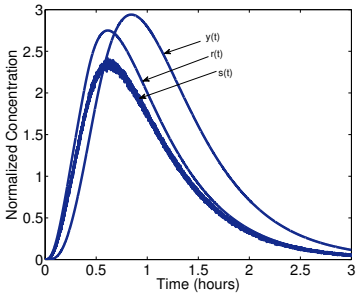


Fig. 20: Concentration of the transmitted signal r(t), the received signal with noise s(t), and the filtered signal y(t).

### B. Receiver

The signal $r(t)$ propagates through a diffusion based channel and it is distorted by the channel noise assumed to be an Additive White Gaussian with zero mean and 0.05 $\mu$M variance [27]. In Fig. 20, both the signal given to the channel, $r(t)$, and the distorted signal, $s(t)$, are shown. Furthermore, the output of the receive filter $y(t)$ shown in Fig. 20 compensates for the attenuation in the channel and filters the noise.

The filtered received signal $y(t)$ is measured by the detector described in Fig. 17 which selects the symbol $'3'$. Then, it generates the corresponding bit sequence '11', i.e., the original information sent by the transceiver A, as shown in Fig. 21. Note that the generated bits do not have perfectly rectangular shapes but the existence of two different peaks indicates the generation of two consecutive '1' bits.

## VIII. Conclusion

In this paper, a biotransceiver design for MC using genetically engineered bacteria is presented. First, the operation principles of biological circuits are modeled with RRE and both the digital and analog interpretations of biological circuits are provided. Then, the interconnections of basic biological circuits are defined. Using this mathematical framework, a biotransceiver architecture tailored for MC environment is proposed. The biological circuit designs for every block of the proposed biotransceiver are presented. Numerical results show that biotransceivers have long delays and very low operation speeds. However, they can operate in parallel within the small cell volume. Therefore, genetically engineered bacteria can achieve a very high computational density in terms of the number of operations per time per volume [15]. Furthermore, since the human body already contains billions of bacteria, usage of genetically engineered bacteria as nanocommunication devices inside human body will reduce biocompatibility problems. Thus, genetically engineered bacteria is a perfect candidate for biomedical applications of nanocommunication networks such as diagnosis and treatment of lethal diseases, health monitoring, drug delivery, bio-hybrid implants.

## Appendix

A RRE model is composed of n chemical species $\{S_1, ..., S_n\}$ and $m$ chemical reactions $\{R_1, ..., R_m\}$. Each reaction $R_j$ has the following form:

$$v_{1j}^r S_1 + ... + v_{nj}^r S_n \underset{k_r}{\overset{k_f}{\rightleftharpoons}} v_{1j}^p S_1 + ... + v_{nj}^p S_n,$$

where in reaction $R_j$, $v_{ij}^r$ denotes the stochiometry for $S_i$ as a reactant and $v_{ij}^p$ denotes the stochiometry for $S_i$ as a product. When $S_i$ participates to a reaction neither as a reactant nor as a product, then $v_{ij}^r$ and $v_{ij}^p$ are assigned 0. The forward rate constant is denoted by $k_f$ while the reverse rate constant by $k_r$. If the reaction is irreversible, then $k_r$ is 0.

According to the law of mass action, the rate of a reversible reaction is proportional to the product of the concentrations

of reactant molecules reduced by a value proportional to the product of the concentrations of product molecules. Hence, the reaction rate $V_j$ for reaction $R_j$ is defined as

$$V_j = k_f \prod_{i=1}^{n} [S_i]^{v_{ij}^r} - k_r \prod_{i=1}^{n} [S_i]^{v_{ij}^p}, \quad (30)$$

where $[S_i]$ is the concentration of species $S_i$. Then, RRE model can be constructed as

$$\frac{d[S_i]}{dt} = \sum_{j=1}^{m} v_{ij} V_j, \quad 1 \leq i \leq n, \quad (31)$$

where $v_{ij} = v_{ij}^p - v_{ij}^r$ (i.e., the net change in species $S_i$ due to reaction $R_j$). RRE model devises one differential equation for each species which is equal to the sum of the rates of change of the species due to each reaction.

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**Bige D. Unluturk** (S'12) received the B.Sc. and M.Sc. degrees in Electrical and Electronics Engineering from Middle East Technical University, Turkey, in 2011 and from Koc University, Turkey in 2013, respectively. Currently, she is a PhD student with the Broadband Wireless Networking Lab at Georgia Institute of Technology, Atlanta, GA under the supervision of Prof. Dr. Ian F. Akyildiz. Her current research interests include nanoscale communications and molecular communication networks.

**A. Ozan Bicen** (S'08) received the B.Sc. and M.Sc. degrees in Electrical and Electronics Engineering from Middle East Technical University, Turkey, in 2010 and from Koc University, Turkey in 2012, respectively. He is currently a Graduate Research Assistant in the Broadband Wireless Networking Laboratory and pursuing his Ph.D. degree at the School of Electrical and Computer Engineering, Georgia Institute of Technology, Atlanta, GA. His current research interests include molecular communication and nanonetworks.

**Ian F. Akyildiz** (M'86-SM'89-F'96) received the B.S., M.S., and Ph.D. degrees in Computer Engineering from the University of Erlangen-Nurnberg, Germany, in 1978, 1981 and 1984, respectively. Currently, he is the Ken Byers Chair Professor in Telecommunications with the School of Electrical and Computer Engineering, Georgia Institute of Technology, Atlanta, the Director of the Broadband Wireless Networking Laboratory and Chair of the Telecommunication Group at Georgia Tech. Dr. Akyildiz is an honorary professor with the School of Electrical Engineering at Universitat Politecnica de Catalunya (UPC) in Barcelona, Catalunya, Spain and founded the N3Cat (NaNoNetworking Center in Catalunya). Since September 2012, Dr. Akyildiz is also a FiDiPro Professor (Finland Distinguished Professor Program (FiDiPro) supported by the Academy of Finland) at Tampere University of Technology, Department of Communications Engineering, Finland. He is the Editor-in-Chief of Computer Networks (Elsevier) Journal, and the founding Editor-in-Chief of the Ad Hoc Networks (Elsevier) Journal, the Physical Communication (Elsevier) Journal and the Nano Communication Networks (Elsevier) Journal. He is an IEEE Fellow (1996) and an ACM Fellow (1997). He received numerous awards from IEEE and ACM. His current research interests are in Terahertz band communication, nanonetworks, software defined networking, 5G cellular systems and wireless underground sensor networks.