Molecular Communications Pulse-based Jamming Model for Bacterial Biofilm Suppression

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***Abstract*—Studies have recently shown that the bacteria survivability within biofilms is responsible for the emergence of *superbugs*. The combat of bacterial infections, without enhancing its resistance to antibiotics, includes the use of nanoparticles to quench the *quorum sensing* of these biofilm-forming bacteria. Several sequential and parallel multi-stage communication processes are involved in the formation of biofilms. In this paper, we use proteomic data from a wet lab experiment to identify the communication channels that are vital to these processes. We also identified the main proteins from each channel and propose the use of jamming signals from synthetically engineered bacteria to suppress the production of those proteins. This biocompatible technique is based on synthetic biology and enables the inhibition of biofilm formation. We analyse the communications performance of the jamming process, by evaluating the path loss for a number of conditions that include different engineered bacterial population sizes, distances between the populations and molecular signal power. Our results show that sufficient molecular pulse-based jamming signals are able to prevent the biofilm formation by creating lossy communications channels (almost -3 dB for certain scenarios). From these results, we define the main design parameters to develop a fully operational bacteria-based jamming system.**

***Index Terms*—Communications systems, Jamming, Synthetic logic circuits, Biofilm suppression**

I. Introduction

In recent years, the field of molecular communications has received considerable interests [1]–[3]. Its objective is to develop communications systems that are constructed from molecular biological components and systems found in nature [1], [3], [4]. One approach that has been proposed uses bacteria to perform molecular communications as carriers, transmitters or receivers. This includes the application of their motility behaviour to deliver DNA-encoded information [5]–[7], or engineering their diffusion-based signalling process between the cells [5], [7], [8]. For this case, the use of the bacteria signalling system (a.k.a *quorum sensing* – QS) allow communications engineers to synthetically coordinate the emission of molecules, whereby in a natural setting allow bacteria to perform certain functions, such as the formation of biofilms [9]. The coordination created by the QS signalling initiates a cascaded communications process within bacteria that lead to these behaviours.

Biofilms can lead to numerous resistant infections within the human body as well as environmental contamination. This is largely due to the protective surface that encapsulates the bacteria to allow them to survive through varying environmental conditions [10]. A recent concern has been raised about the role that biofilms have in protecting the bacteria from antibiotics. This protective mechanism has led to the emergence of the *superbug*, which are bacteria that have resistance to all current types of antibiotics [11]. Besides health issues, biofilms are also known to impact the environment, where they have led to contamination of fruits, vegetables and drinking water distribution systems [12], [13]. The formation of biofilms is triggered by a multi-stage communications process. Initially, bacteria will search for a suitable and favourable environment to form biofilms [14] (see Figure 1a). Once this location is found, bacteria will form a biofilm infrastructure that surrounds them and start to perform complex internal communication processes that help to maintain the whole infrastructure. This infrastructure is known as the *Extracellular Polymeric Substances* (*EPS*) matrix [15]. Numerous efforts have been dedicated for the dysregulation of bacteria resistance mechanisms [16], [17] and for the eradication of biofilms, including the use of chemical agents or synthetically engineered mechanisms [18]–

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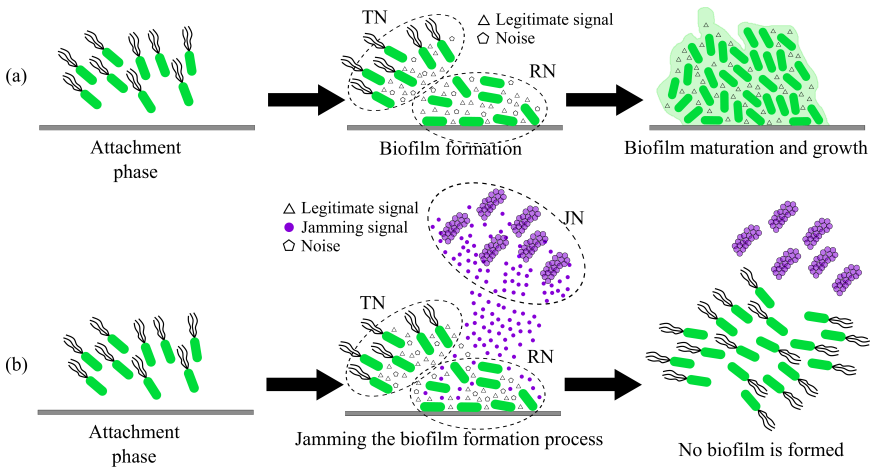


Fig. 1. A general illustration of the biofilm formation process in two different scenarios. (a) The biofilm bacteria within the transmitter node TN will produce different molecular protein signals to induce the receiver bacterial population to form the biofilm. (b) The case where engineered jamming bacteria node JN produces molecular protein signals that will interfere with the multi-stage communication process between TN and RN biofilm bacteria, inhibiting the biofilm formation.

[20]. In this paper, we will investigate an approach for preventing biofilm to form, based on interfering with its multi-stage and parallel communication processes.

We propose the use of synthetically engineered bacteria to transmit diffusion-based jamming signals to a group of bacteria that are aiming to produce a biofilm. The proposed model is based on jamming systems used in conventional wireless networks. This analogy mapping is illustrated in Figure 1b, where the engineered bacteria within the jamming node (JN), through the QS process, will emit concentrations of proteins to disrupt the communication process of the biofilm forming bacteria (contained within the transmitter node TN and receiver node RN). We focus on **how the interaction and communications process** between two bacterial populations, **will prevent the formation of the biofilm**. The objective of the molecular pulse based jamming signal is to degrade the performance (obtaining a high path loss) of the TN and RN bacteria communications link.

The modelling of molecular communications interference process has been proposed before [21]. However, there is no application of using natural bacteria signalling for disrupting molecular communications systems, and in particular for a biotechnology application, such as bacterial biofilm suppression. Our pulse based jamming model includes emulated digital toggle switches for the RN bacterial population, that change between states depending on a specific induction signal. For our model, we consider that **the molecular pulse-based jamming signal will induce the production of proteins that inhibit the biofilm formation**. We identified a number of channels used in the communication process for the biofilm formation and modelled each one as toggle switches [22]–[27]. The aim is to force the induction of suppressor molecules as the molecular pulse-based jamming signal that will prevent the formation of bacterial biofilms. We integrate the use of synthetic biology and molecular communications to develop this biocompatible solution. The main contributions of this paper are:

- **Identification of weak points within the biofilm formation multi-stage communications** to interfere using a molecular pulse-based jamming signal. We identify three main internal communication channels and their corresponding signalling proteins. For this paper, we used raw mass spectrometry proteomics data collected from wet lab experiments.
- **Design of a pulse-based jamming model to interfere an end-to-end bacteria-based molecular communications systems.** We use a bacterial population to emit *quorum sensing* molecules to disrupt and lower biofilm-related protein production. The path loss metric is used to determine the performance of the proposed model.
- **Actuation on a bacteria natural behaviour using synthetic biology and molecular communications.** The effectiveness of the proposed interfering system is dependent on the number of jamming molecules that reach the bacterial population and disrupt its intracellular signalling system. We emulate the internal bacterial process of activation (or deactivation) of the molecules to create the biofilm as toggle switches. The switching mechanism is then controlled by the molecular communications of proteins emitted by the engineered jamming

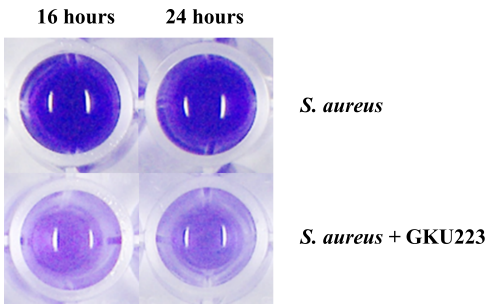


Fig. 2. Wet-lab demonstration of the *S. aureus* biofilm formation in a 96-well microtiter plate. The top row shows the isolated bacteria, leading to the biofilm formation. The bottom row shows the *S. aureus* after being treated with supernatant of *Streptomyces sp. GKU 223*, preventing the biofilm formation. Both cultures were cultivated in the 96-well microtiter plate at 37°C for 16 and 24 hours. The biofilm mass was stained using crystal violet. This result shows that the biofilm formation can be prevented based on applying supernatant, which in turn suppresses certain communication channels.

bacteria.

The rest of the paper is organised as follows. In Section II, we describe the *Staphyloccocus aureus* biofilm formation as a communications system and how it can be engineered to promote and suppress biofilm-related proteins. In Section III we present the physical model of the bacteria-based molecular communications system and the pulse-based jamming system. The analysis of different interfering scenarios for the proposed communications system is presented in Section IV. Lastly, in Section V we present our conclusions.

II. Background on Communications Process for Biofilm Formation

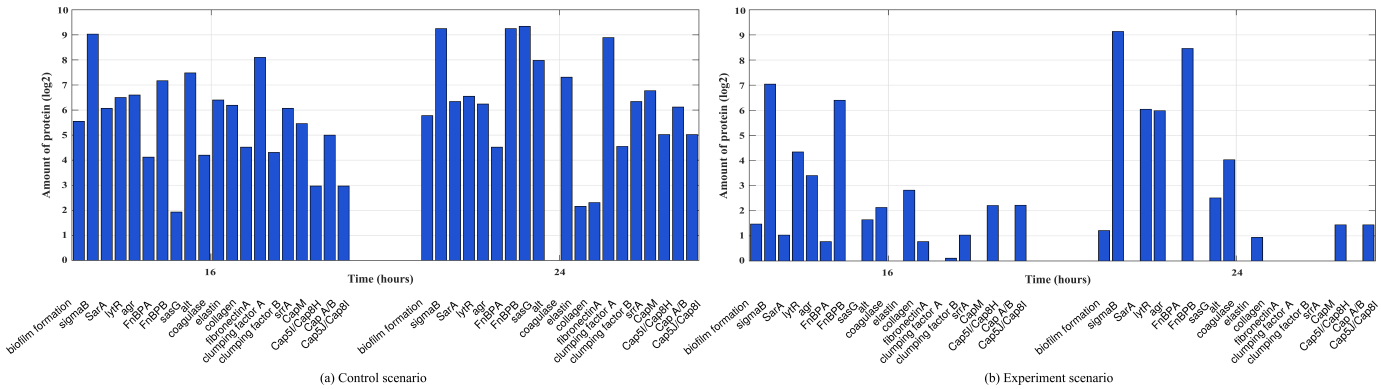
In order to determine the multi-stage communication process of biofilms, as well as deducing the types of proteins used in the signalling, wet lab experimental work was conducted. In the experiments, *Staphylococcus aureus* (we will refer to this as *S. aureus* for the remainder of the paper) was used as a biofilm-producer. Proteomic data were collected and compared between the stages during the full formation, and the case when a chemical agent is applied to prevent the formation. The proteomic data was deposited to jPOST (http://repository.jpostdb.org/, ID: JPST000480 and PXD010815). This chemical agent can be produced by a separate species known as a marine *Streptomyces sp.* GKU 223 [29]. Figure 2 demonstrates this comparison, where we can observe the difference in the biofilm mass after 16 and 24 hours when *S. aureus* was grown on its own (leading to biofilm formation) versus the case when biofilm was inhibited when in contact with the active chemicals produced by *Streptomyces sp.* GKU 223. Figure 3 shows the protein expression level of capsular biosynthesis, cellular stress, virulence, cell viability, and biofilm formation of *S. aureus*. The obtained data (see Figure 3) showed three different sets of proteins (*i.e.*, internal communications channels) that directly facilitated the multi-stage communication process for the biofilm formation. The internal communication channels are comprised of *cellular defence channel*, *cellular stress response channel*, and *energy supply channel*. For each of these channels, the proteins are triggered during different stages of the biofilm construction, as shown in Figure 4. Therefore, the objective of this paper is to piece out the relevant proteins for each of the channels and to disrupt their production through the jamming process. Detailed description for each of the channels will be presented in this section, as well as a description of the emulated toggle switch that controls the intra-cellular signalling for the protein production.

A. Cellular Defence Channel

Bacteria constantly monitor and respond accordingly to environmental changes. They coat their membranes using *Capsular Polysaccharides* (*CPS*) as a defence mechanism to these changes [22]. Each bacterial species can synthesize a number of different *CPS* serotypes. For example, *S. aureus* can synthesize up to thirteen different serotypes, which provide a better defence mechanism when compared to other bacterial strains [22]. Figure 5 presents the communications system involved in the *CPS* synthesis for *S. aureus*. The *CPS* starts to be produced when the bacteria sense specific environmental cues, including the culture medium's pH, iron limitation and CO_2 availability [10], [23]. These environmental signals trigger the expression of *CapM*, which induces the cascade production of other capsular polysaccharides (*CapL*, *Cap5I/Cap8H*, *Cap A/B* and *Cap5J/Cap8I*). Therefore, targeting the suppression of *CapM* will affect the entire defence channel and prevent biofilm formation [24].

B. Cellular Stress Channel

In response to cellular stress, bacteria can produce a matrix of *Extra Polymeric Substances* that protects them from physical attacks [25]. One of main components of the matrix is the *Polysaccharide Intercellular Adhesin* (*PIA*) [25]. In *S. aureus*, the *Agr quorum sensing* system induces the production of *PIA* mediated by *Staphylococcus acessory regulator A* (*SarA*) and *ica* pathway. The activation of the global regulator *SarA* also induces the production of other *Microbial Surface Components Recognizing Adhesive Matrix Molecules* (*MSCRAMMs*) like



(a) Control scenario (b) Experiment scenario

Fig. 3. A highlight of the most significant 19 proteins extracted from more than 1500 different types that were detected in the two experiment cases (*S. aureus* on its own and *S. aureus* mixed with the supernatant of *Streptomyces sp. GKU 223*). These concentration levels are important for characterising the three bacterial internal communication channels that are essential towards biofilm formation and maintenance: defence, energy supply and cell stress response.

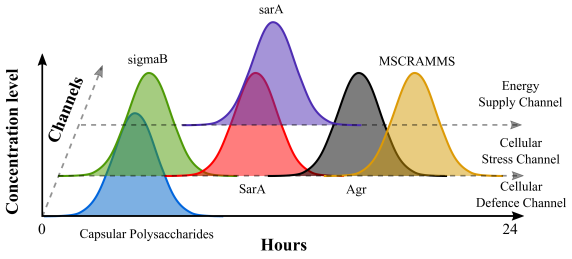


Fig. 4. Temporal illustration of the key proteins produced for the cellular defence, cellular stress response, and energy supply communication channel produced by the bacteria during the biofilm formation. Each of the proteins for the three communication channels is triggered at various stages of the biofilm lifetime.

lytR, *FnBPA*, *FnBPB*, *sasG* and *atl* (see Figure 6). These proteins are produced in response to any stress subjected onto the bacterial surface [25]. High concentration levels of these proteins within the bacterial cell will result in stronger adhesion to the substrate, as well as to other cells. Therefore, low levels of adhesins produced by *SarA* suppression can contribute towards the disassembling of the biofilm matrix structure. This particular protein molecule will be targeted for the jamming process.

C. Energy Supply Channel

The gene responsible for the respiratory response (*srrA*) in *S. aureus* acts as a global regulator of virulence factor and also has an important role in biofilm formation [26]. In environments with low oxygen, *srrA* is activated and promotes cellular adaptation for bacterial population maintenance (see Figure 7). In this case, the energy supply is provided through fermentation using the *ica* pathway and enzymes (*nrdD* and *nrdG*) [26]. However, low levels of *srrA* can lower the biofilm energy supply to a minimum level and contributes towards the biofilm disassembling process [27], which will also be another targeted protein for our proposed jamming process.

D. Toggle Switches Emulation

A molecular pulse-based jamming signal will trigger the production of specific suppressors, in order to reduce the production of the targeted proteins in each channel. This process can be emulated through the activation of toggle switches. In order to achieve this, we abstracted the protein production processes $h_{D1}(t)$, $h_{S1}(t)$ and $h_{F1}(t)$ (shown in Figure 5-7) as toggle switches that will trigger a cascade effect for each internal communication channel. As illustrated in Figure 8, the identified proteins *CapM*, *SarA*, *srrA* (target points along the communication process) will be one input for each of the toggle switches. Complementing these proteins, we have identified three other proteins that can be used as the second input to control the toggle switch. Effectively, these second inputs will lower the levels of the first inputs. The jamming signal will induce the production of *KdpDE*, interfering with *CapM* production, which has been suggested to decrease the transcription level of *CPS* [24]. In the case of the cellular stress toggle switch, the jamming signal will induce *SarR* production, which is a repressor for *SarA* production [30], and, for the energy supply toggle switch, *srrA* can be repressed by the induction of *pYJY4* protein [27].

We consider a fast and reversible binding process between the molecular pulse-based jamming signals and the bacterial receptors. The toggle switches will be induced by the jamming signal as shown in Figure 8. Therefore, each switch can be modelled based on the following Hill functions [31]

$$\frac{dX}{dt} = \frac{\beta}{1 + K_Y^{\alpha}} - \gamma X \tag{1}$$

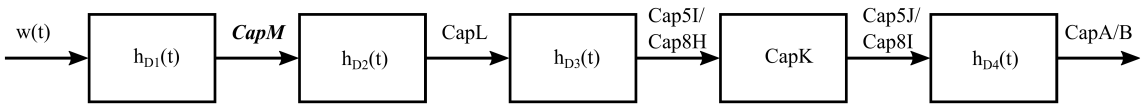


Fig. 5. The sequential stages of protein production, and their corresponding channel, during the cellular defence communications system. The selected protein highlighted in bold, *CapM*, is the molecular pulse-based jamming target. The signal $w_d(t)$ is the input signal that triggers the defence channel communication cascade

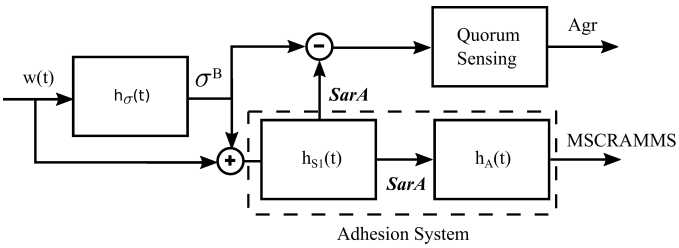


Fig. 6. The sequential and parallel stages of protein production, and their corresponding channel, during the cell stress response communications system (please note that certain channels are results of protein summations from previous stages). The selected protein highlighted in bold, *SarA*, is the molecular pulse-based jamming target. The signal $w_s(t)$ is the input signal that triggers the stress channel communication cascade.

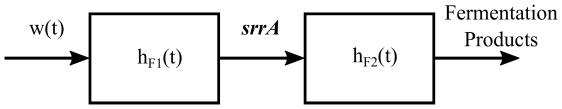


Fig. 7. The sequential stages of protein production, and their corresponding channel, during the cell energy production communications system. The selected protein highlighted in bold, *srrA*, is the molecular pulse-based jamming target. The signal $w_e(t)$ is the input signal that triggers the energy channel communication cascade

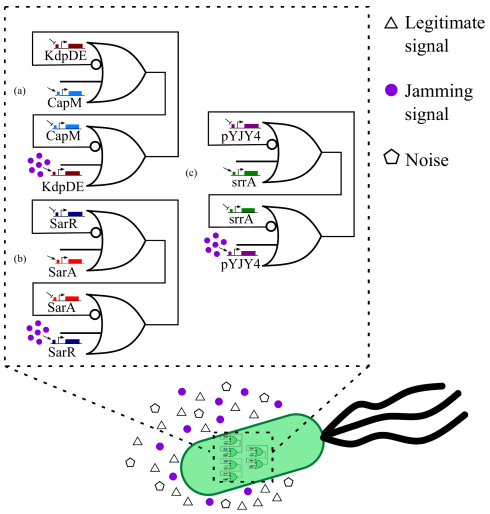


Fig. 8. The targeted protein production of *CapM*, *SarA*, *srrA* for the three internal communication channel, and its control can be represented as toggle switches. (a) The cellular defence system can be triggered by an increasing level of *CapM* and deactivated by an increasing level of *KdpDE*. (b) For the cellular stress response communications, *SarA* is deactivated by an increased level of *SarR*. (c) For the energy system, *srrA* is deactivated by an increased level of *pYJY4*.

and

$$\frac{dY}{dt} = \frac{\beta}{1 + K_X^{\alpha}} - \gamma Y \tag{2}$$

where X represents the protein levels of *CapM*, *SarA* or *srrA*, Y represents the protein levels of *KdpDE*, *SarR* or *pYJY4*, β is the maximum production rate for the selected inputs; α is the repression constant for the promoters X and Y, w_Y and w_X are the molecular pulse-based jamming signals that induce the toggle switches to work, $K_X = X/(1+(w_X/K)^n)$ and $K_Y = Y/(1+(w_Y/K)^n)$ are binding constants, γ is the first-order decay constant and n is the Hill coefficient.

III. The Physical Model

The previous section described how biofilm inhibition can be achieved by suppressing specific proteins, and how this could be achieved through a toggle switch model that controls the intracellular signalling pathways controlling the protein production. Therefore, from a communication systems perspective, we must develop a model that will lead to the production of molecular pulse-based jamming signals that will interfere with the three internal communications channels, and suppressing the production of the targeted proteins. Our objective is to ensure the molecular protein jamming signals will reach the receiver biofilm bacteria population, bind to the bacterial membrane surfaces and block the cascade of sequential protein production events that will result in the inhibition of biofilm formation.

A general perspective of the proposed molecular communications system is presented in Figure 9. We consider a finite 2D aqueous environment with two bacterial populations (to recreate the environment of the wet-lab experiment). One will act as transmitter and receiver biofilm bacteria (placed in the transmitter and receiver nodes), TN and RN respectively and the other will be the engineered bacteria placed in the jamming node JN. The transmitter node TN is composed of n_t bacteria which will send a signal to the receiver node RN (with n_r bacteria). The jamming node JN, with n_j bacteria,

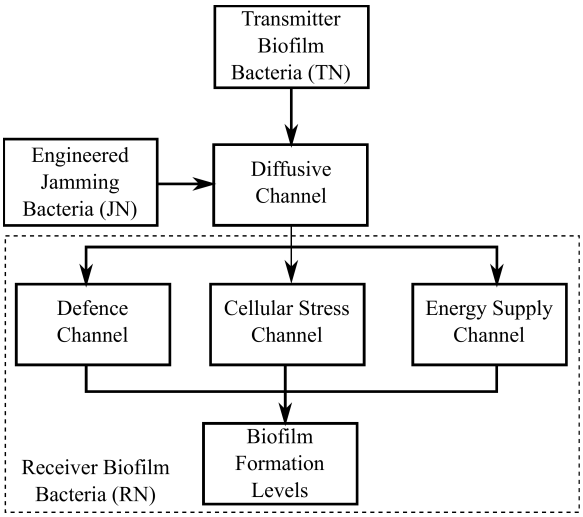


Fig. 9. Molecular communications system model to disrupt the biofilm formation.

will produce signals that influence the toggle switches of the receiver node RN bacteria in order to disrupt their internal communications channels (as presented in Section II). Each bacterium in TN and JN is considered as an emitting point source and is placed at random locations within a circle of radius r_{TN} and r_{JN} from the centre of the receiver node. Both the biofilm forming signalling molecules, as well as the jamming signalling molecules, propagates through free diffusion in the aqueous medium. We ignore the effects of collision that can occur among the molecules during the diffusion process.

The biofilm bacteria in the transmitter node are responsible for producing and diffusing a signal to induce the behaviour of the bacteria population in the receiver node to form a biofilm. The signal, denoted by $A_{TN,e}(\hat{t})$, is the QS signalling produced by the transmitter biofilm bacteria population. The jamming signal, $A_{JN,e}(\hat{t})$, is the time-related concentration of the proteins capable of activating the toggle switch for each internal communications channel of the receiver bacteria. In our model, both the communication for the biofilm formation and the molecular pulse-based jamming signal are described using the following set of differential equations [32]:

$$\frac{dA_m(\hat{t})}{dt} = c_A + \frac{k_A C_m(\hat{t})}{K_A + C_m(\hat{t})} - k_0 A_m(\hat{t}) - k_1 R_m(\hat{t}) A_m(\hat{t}) + k_2 R A_m(\hat{t}) - p_{out} A_m(\hat{t}) + p_{in} A_{m,e}(\hat{t}), \tag{3}$$

$$\frac{dR_m(\hat{t})}{dt} = c_R + \frac{k_R C_m(\hat{t})}{K_R + C_m(\hat{t})} - k_3 A_m(\hat{t}) - k_1 R_m(\hat{t}) A_m(\hat{t}) + k_2 R A_m(\hat{t}), \tag{4}$$

$$\frac{dRA_m(\hat{t})}{dt} = k_1 R_m(\hat{t}) A_m(\hat{t}) - k_2 R A_m(\hat{t}) - 2k_4 R A_m(\hat{t})^2 + 2k_5 C_m(\hat{t}), \tag{5}$$

$$\frac{dC_m(\hat{t})}{dt} = k_4 R A_m(\hat{t})^2 + k_5 C_m(\hat{t}), \tag{6}$$

$$\frac{dA_{m,e}(\hat{t})}{dt} = (p_{out} A_m(\hat{t}) - p_{in} A_{m,e}(\hat{t})) - D A_{m,e}(\hat{t}), \tag{7}$$

where $A_m(\hat{t})$, $A_{m,e}(\hat{t})$, $R_m(\hat{t})$, $RA_m(\hat{t})$, $C_m(\hat{t})$ are the internal and external autoinducer, receptor, complex and dimerized complex concentrations, respectively; c_A and c_R are the transcription basal levels for $A_m(\hat{t})$ and $R_m(\hat{t})$, respectively; k_A and k_R are the transcription rates; K_A and K_R are the degradation rates, $k_0 - k_5$ are the translation rates; p_{in} and p_{out} are transport rates inside and outside the bacteria, respectively; $\hat{t} = t - \tau_p$ and τ_p is the production delay; and $m = TN$ is when the molecular signals are emitted by the transmitter biofilm bacteria or $m = JN$ if the molecular signals are emitted by the engineered bacteria.

In absence of the molecular pulse-based jamming signal, the received signal $s(t)$ can be expressed as

$$s(t) = h_t(t) * (n_t A_{TN,e}(\hat{t})) + n(t) \tag{8}$$

where $n(t)$ is the Additive White Gaussian Noise, t is the time in hours and '$*$' denotes a convolution operation [33].

After reaching the receivers, the molecular pulse-based jamming signal will affect the protein production related to the initial steps of each internal communications processes. To interfere with the legitimate transmission, all engineered bacteria in the jamming node JN will diffuse molecules at the same time towards the receiver biofilm bacteria population. However, there are cases where parts of the bacterial population in JN are synchronised, and others are not, compromising the jamming effectiveness (see Figure 10). We evaluate this scenario by considering that the bacteria population in the jamming node JN can have v partitions with each starting to produce jamming molecules at different time periods. Therefore, these partitions can suffer from

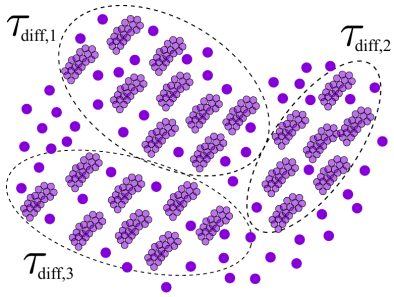


Fig. 10. An illustration of the jamming bacterial population partitions that represent segmented bacterial populations which will diffuse molecules at different time periods. Since in this example $v = 3$, the jamming node JN will have three random $\tau_{d,v}$ values.

different delays in the system and (8) can be represented as

$$s_j(t) = h_t(t) * (n_t[A_{TN,e}(\hat{t})]) + \overbrace{\sum_{i=1}^{v} h_j(t-\tau_{d,v}) * (n_{j,v}[A_{JN,e}(\hat{t})])}^{w(t)} + n(t), \tag{9}$$

where τ_d is the propagation delay for a signal produced by the engineered bacteria in the jamming node JN (in hours), and r_{JN} is the Euclidean distance between the JN and RN populations.

Bacteria diffuse molecules into the environment when signalling between each other [9]. These molecules propagate by Brownian motion and can be represented through Fick's law [34]. The received signal will vary according to time and the distance between the TN-RN and JN-RN populations. The channel for both the transmitter biofilm bacteria and the engineered bacteria can be defined as [35]

$$h_t(t) = \frac{1}{1 + e^{((r_{TN} - vt)/\sqrt{2})}} \tag{10}$$

$$h_j(t-\tau_{d,v}) = \frac{1}{1 + e^{((r_{JN} - v(t-\tau_{d,v}))/\sqrt{2})}} \tag{11}$$

where r_{TN} and r_{JN} are the average Euclidean distances from the centre of the transmitter node TN and jamming node JN to the centre of the receiver node RN and v is the velocity of the wave formed by the molecular pulse-based jamming signal propagation.

To measure the impact of the interference on the legitimate transmission we evaluate the path loss P_L in this system. Using equations (3)-(10), the path loss measured at receiver node RN when there are no engineered bacteria present can be expressed as

TABLE I
PARAMETERS USED FOR THE EVALUATION OF THE EQUATIONS (3)-(13). THE VALUES WERE OBTAINED FROM [32]. THE VALUES FOR β, K, n AND α WERE OBTAINED BY FITTING EQUATION (1) AND (2) TO THE EXPERIMENT RESULTS SHOWED IN FIGURE 3.

Variable	Value	Unit
c_A, c_R	2.7×10^{-2}	nM
k_A, k_R	2×10^{-3}	d^{-1}
k_0	1×10^{-2}	d^{-1}
k_1, k_2, k_4, k_5	0.1	d^{-1}
k_3	1×10^{-2}	d^{-1}
K_A, K_R	2×10^{-3}	gm^{-3}
p_{in}, p_{out}	0.1	d^{-1}
$[A_{TN}]_{initial}$	2	nM
$[R_{TN}]_{initial}$, $[R_{JN}]_{initial}$	0.15	nM
$[RA_{TN}]_{initial}$, $[RA_{JN}]_{initial}$	0	nM
$[C_{TN}]_{initial}$, $[C_{JN}]_{initial}$	0	nM
$[A^e_{TN}]_{initial}$, $[A^e_{JN}]_{initial}$	0.1	nM
β	80000	nM
σ_n^2	1	W
D	4.9	cm^2/h
α	1	–
K	10	–
n	2	–

$$P_L = \frac{P_t}{P_r} = \frac{n_t}{T^2} \int_{t=1}^{T} \frac{|A_{TN,e}(\hat{t})|^2}{|s(t)|^2}, \tag{12}$$

where T is the duration of the transmitted signal. When the engineered bacteria start to produce the molecular pulse-based jamming signal, the path loss P_{LJ} at the receiver for this scenario can be evaluated using equations (3)-(11) and represented as

$$P_{LJ} = \frac{P_{tj}}{P_{rj}} = \frac{2n_t}{T^2} \int_{t=1}^{T} \frac{(|A_{TN,e}(\hat{t})|^2 + |A_{JN,e}(\hat{t})|^2)}{|s_j(t)|^2}. \tag{13}$$

IV. MODEL ANALYSIS

Our interest in this analysis is to obtain the highest attenuation on the legitimate signal by interfering with the communication process leading to the biofilm formation. This will result in an ideal jamming performance. Consequently, more proteins will reach the biofilm receiver bacteria and activate their internal communications channels. This whole cascade will suppress production of biofilm-forming proteins. The parameter values used to evaluate all the equations in this paper are presented in Table I.

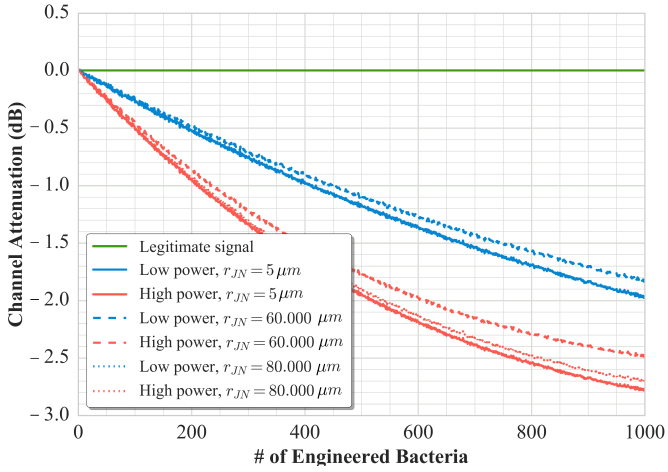


Fig. 11. The path loss in dB for a variable number of engineered bacteria at a fixed distance between JN and RN bacterial populations ($r_{JN} = 5\,\mu$m, $60,000\,\mu$m and $80,000\,\mu$m). There is also no delay associated to both the production and transmission for the legitimate and molecular pulse-based jamming signals, $\tau_d = \tau_p = 0$.

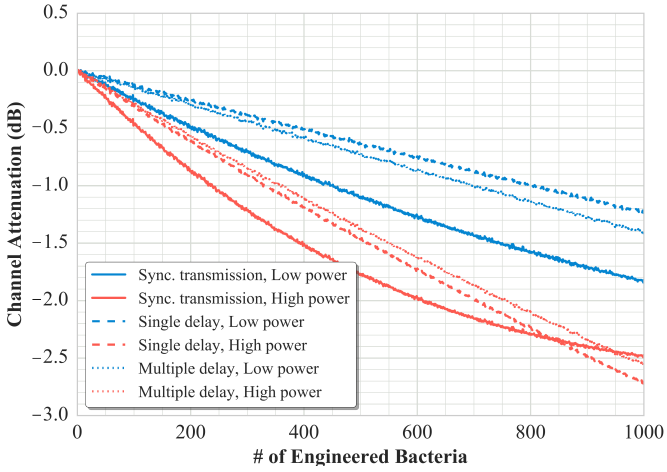


Fig. 12. The channel attenuation when the production of the molecular pulse-based jamming signal is delayed. In this case, the interfering channel has a single propagation delay (for a single partition) and multiple delays (for multiple partitions). We also consider a variable number of engineered bacteria at a fixed distance to the receiver node RN.

A. Path Loss Analysis

In this section, we analyse the impact of the molecular pulse-based jamming signals from the engineered bacteria on the TN channel. We analysed equations (12)-(13) by varying the number of engineered bacteria in the jamming node (n_j ranging from 1 to 1000), where each JN bacterium could produce two distinct jamming signals ($[A_{JN}]_{initial} = 0.2$ nM and $[A_{JN}]_{initial} = 1$ nM). We considered that the engineered bacteria population in the jamming node is composed by one single partition ($v = 1$), there is no delay for the production and propagation for the legitimate and interfering signals ($\tau_d = \tau_p = 0$), three different JN-RN distances ($5\,\mu$m, $60.000\,\mu$m and $80.000\,\mu$m), both the legitimate and molecular pulse-based jamming signals were produced within 16 hours and the noise power was considered as $\sigma_n^2 = 1$.

It can be observed from Figure 11 that the attenuation increases proportionally with the number of engineered bacteria. The power of a molecular pulse-based jamming signal also affect the path loss for this system. A greater attenuation is produced by the higher power molecular signal when compared with the lower power. Despite the variations caused by the power of the molecular pulse-based jamming signal, the noise does not produce a strong effect on the path loss and this is due to the low power level ($P_n = 1W$). Figure 11 shows that the path loss can also vary according to the average distance between the engineered and biofilm receiver bacteria r_{JN}. However, small variations in the average distance r_{JN} do not produce noticeable attenuation values. For this particular scenario, within the considered observation time, most of the protein concentration diffused by the engineered bacteria is able to reach the biofilm bacteria receiver. Therefore, they almost do not differ from the case when $r_{JN} = 5\,\mu m$. From this result, we can see that both the average distance r_{JN} and the power of the molecular pulse-based jamming signal are important parameters to ensure a higher interference requirement on the bacterial internal communications channels.

Next, we analyse the impact of both the production and propagation delays in the path loss. We consider an average JN-RN distance ($r_{JN} = 60.000\,\mu$m) and vary the number of engineered bacteria during an observation window of 16 hours. In this case, the propagation delay $\tau_{d,v}$ is produced by the diffusive medium for each partition. Without loss of generality, we considered the propagation delay $\tau_{d,v}$ is random and range from 0 to 4 hours. In Figure 12, we can observe the comparison between the synchronised transmission scenario and when the single and multiple partitions are subjected to random propagation delay values ($\tau_{d,1} = 3.53$ hours, $\tau_{d,2} = 3.94$ hours, $\tau_{d,3} = 1.63$ hours, $\tau_{d,4} = 0.20$ hours). For the single and multiple partitions curves presented in Figure 12 the *quorum sensing* production is delayed by $\tau_p = 3$hours.

The single partition case ($\tau_{d,1} = 3.53$ hours), with a low molecular pulse-based jamming signal power, results in a lower path loss, when compared to all other cases. On the other hand, for a high molecular pulse-based jamming signal power, the same single partition produces

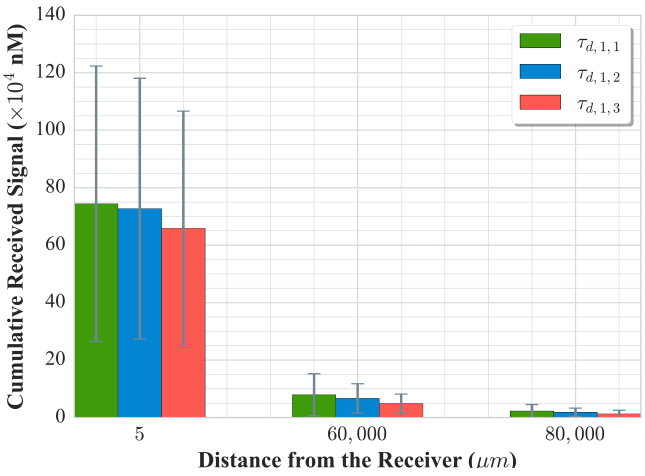


Fig. 13. The effect of the propagation delay $\tau_{d,v}$ on the path loss for a single partition transmission ($\tau_p = 3$ hours). We also considered a fixed number of engineered bacteria ($n_j = 200$) and three different distances to the receiver node values.

a higher path loss. When subjecting this communication system to multiple delays, the path loss is similar to the single partition case, for both low and high power interfering with molecular signals. The worst path loss occurs when the system is affected by a synchronised molecular pulse-based jamming signal. However, is important to note that is possible to achieve a worse path loss scenario if using a higher number of engineered bacteria ($n_j \geq 800$) and a high power molecular pulse-based jamming signal. These results show that the molecular pulse-based jamming signal power can compensate the effects of a single or small delays. They also highlight the importance of using synchronised transmissions to obtain a higher interference.

For the last analysis of this communication system path loss, the following conditions are considered: three r_{JN} average distances were considered (5 μm, 60,000 μm, 80,000 μm) and a single partition composed by $n_j = 200$ engineered bacteria producing a delayed low power molecular pulse-based jamming signal ($\tau_p = 3$ hours) which is subjected to three propagation delays ($\tau_{d,1,1} = 2.28$ hours, $\tau_{d,1,2} = 2.50$ hours and $\tau_{d,1,1} = 3.16$ hours). Figure 13 shows that when subjected to the lower propagation delay $\tau_{d,1,1}$, more molecular pulse-based jamming signal is able to reach the receiver. The opposite effect occurs for the higher propagation delay value $\tau_{d,1,3}$. It also can be seen in Figure 13 that these results are independent of the distance from the receiver r_{JN}, despite affecting on the total amount of received molecular pulse-based jamming signal. For example, there is a reduction of more than 90% when comparing the shortest and the longest distances (r_{JN}) considered in this analysis.

Figures 11-13 also shows that both the propagation delay and the average distance between the engineered bacteria and the biofilm bacteria receiver are important factors for the design of this communication system. The highest path loss value is achieved for the case of synchronised molecular pulse-based jamming signals, which means that a lower concentration of protein is able to reach the RN bacterial population and activate the defence, stress and energy communications channels. Consequently, to ensure the high interference needed to suppress the biofilm formation, a shorter r_{JN} distance and a lower propagation delay, either for single or multiple sources of interference, are fundamental.

B. Toggle Switch Activation Analysis

In this section, we analyse the toggle switch activation performance based on the results presented in Section IV-A. To activate a toggle switch, a certain amount of molecular pulse-based jamming signal is required to reach the RN bacterial population and induce the production of the biofilm suppression proteins (as described in Section II, each internal communication channel has a target protein to be induced). Since the total amount of received molecular pulse-based jamming signal is important to trigger the switches, we conducted two analyses. In all cases, the number of engineered jamming bacteria and the number of receiver biofilm bacteria are fixed ($n_j = 1000$ and $n_r = 1000$, respectively).

First, we evaluate the amount of pulse-based jamming signal molecules that reach the receiver node within 16 hours. In this case, we assume a fixed distance between the engineered bacteria and the receiver nodes (r_{JN}), synchronised transmission, a single partition subjected to propagation delay $\tau_{d,1} = 0.33$ hours, and multiple partitions subjected to propagation delay $\tau_{d,1} = 0.33$ hours, $\tau_{d,2} = 3.29$ hours, $\tau_{d,3} = 0.23$ hours and $\tau_{d,4} = 0.51$ hours. As seen in Figure 14, the synchronised transmission is the most efficient in reaching the toggle switch threshold (around 8.5hours). However, this result is not so different when compared with the time taken by both the single and multiple partitions (around 8.7hours and 9hours, respectively). We emphasise that the results shown in Figure 14 are dependent on the propagation delay values. It is possible that a single partition reaches the toggle switch threshold later when compared to the multiple partition. However, this is not very likely to occur.

The cumulative molecular pulse-based jamming signal reception for a synchronised and delayed transmission is plotted against the toggle switch activation threshold.

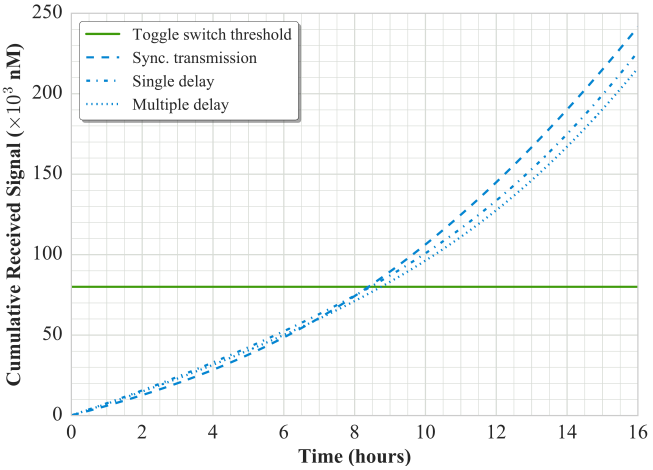


Fig. 14. The cumulative molecular pulse-based jamming signal reception for a synchronised and delayed transmission is plotted against the toggle switch activation threshold.

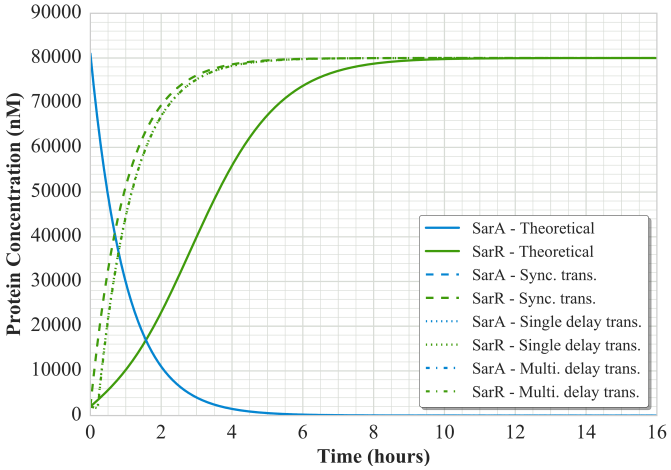


Fig. 15. Toggle switch activation of the cellular stress channel for three different scenarios. For each scenario, a few parameters were modified to observe the different toggle switch behaviours. The parameters used to evaluate these plots are presented in Table I.

Second, we evaluate how fast the received molecular pulse-based jamming signal induces the production of biofilm suppressors. In the previous analysis, we identified that both the production and propagation delays have an impact on the amount of pulse-based jamming signal molecules that reach the receiver node. Furthermore, for both the synchronised and the single partition transmissions, more pulse-based jamming signal molecules were able to reach the toggle switches activation threshold. Therefore, to perform this second analysis, using the same parameter values of Figure 14, we fit the received protein concentration of the previous analysis as two quadratic functions (for the single partition as well as for the multiple partition scenarios) and use it to induce the toggle switches. The other parameters needed to evaluate Equations (1) and (2) are obtained from the experimental data presented in Figure 3, including the starting values for X and Y (81 nM and 2 nM, respectively). For this analysis, we define the activation time as the period taken by the suppressor concentration to become higher than the biofilm formation protein. Figure 15 shows the theoretical curve obtained for the cellular stress communications channel considering a fixed inducer concentration and plotted against the synchronised and delayed molecular pulse-based jamming signal transmissions. There is almost no difference between single and multiple delay transmission activation time. Therefore, in Figure 15 we are only able to compare the theoretical curve with the synchronised and delayed transmissions. As seen in Figure 15 the theoretical activation of the toggle switch occurs around 1.5 hours and takes more than 6 hours for the suppressor signal to reach its maximum value (this is the green solid curve). The fastest toggle switch activation occurs when the molecular pulse-based jamming signal is transmitted without delays. However, the difference between the synchronised and delayed cases is small, where the delayed transmission activates 12 minutes later compared to the case of the synchronised transmission. Additionally, the reducing of the biofilm formation proteins takes the same amount of time in all cases observed. This result suggests that despite the delays can disrupt the communication process related to the biofilm formation, it is not strong enough to affect the activation of the toggle switches and, consequently, it demonstrates the robustness of the proposed molecular pulse-based jamming system. Please note that we only analyse the toggle activation for the cellular defence channel as other two bacterial internal communications channels, namely energy and defence channels, produced similar results to the ones shown in Figure 15.

V. Conclusion

Biofilms are responsible for a number of chronic infections, and numerous solutions are continuously being researched to curb their formation. Since this system involves a multi-stage communication process between the bacteria, we show that biofilm formation can be disrupted by applying a molecular pulse-based jamming signal. Based on wet lab experiments, we were able to identify three internal communication channels that lead to the biofilm formation. For each of them, we identified key proteins that need to be targeted for the jamming process. We show that the molecular pulse-based jamming signal degrades the biofilm bacteria

communication to a level that is sufficient to interfere with the biofilm-related protein production, represented by emulated toggle switches. This paper presented an analysis for a number of parameters that include the difference in population between the jamming as well as biofilm-forming bacteria, the distances between these populations and the power of the molecular pulse-based jamming signal from the engineered bacteria. We also analysed the impact caused by the different number of partitions and their respective production and propagation delays on the amount of pulse-based jamming signal molecules arriving at the receiver node RN. Our results suggest that the average distances between the jamming and receiver nodes, as well as the delays that the molecular pulse-based jamming signal is subjected to, have a high impact on the communications process that leads to the biofilm formation. The obtained results lay the foundation for key design parameters that are needed to ensure a fully operational bacteria-based molecular communications jamming system, and this includes: engineering the JN bacterial population and placing them at a strategic distance to the biofilm-forming bacteria, ensuring synchronisation between the jamming signal transmission and molecular signalling between the TN and RN bacterial populations, and synchronising the engineered jamming bacterial population molecular signal production to ensure that sufficient concentration is produced without stressing the engineered bacteria.

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