Physical Channel Characterization for Medium-Range Nano-Networks using Flagellated Bacteria

Maria Gregori[b],[**], Ignacio Llatser[a], Albert Cabellos-Aparicio[a],[*], Eduard Alarcón[a]

[a]*NaNoNetworking Center in Catalonia (N3Cat, http://www.n3cat.upc.edu), Universitat Politècnica de Catalunya, c/Jordi Girona, 1-3, 08034 Barcelona, Spain*
[b]*Department of Computer Architecture, Universitat Politècnica de Catalunya, c/Jordi Girona, 1-3, 08034 Barcelona, Spain*

Abstract

Nano-networks are the interconnection of nano-machines and as such expand the limited capabilities of a single nano-machine. Several techniques have been proposed so far to interconnect nano-machines. For short distances (nm-mm ranges), researchers are proposing to use molecular motors and calcium signaling. For long distances (mm-m), pheromones are envisioned to transport information. In this work we propose a new mechanism for medium-range communications (nm-μm): *flagellated bacteria*. This technique is based on the transport of DNA-encoded information between emitters and receivers by means of a bacterium. We present a physical channel characterization and a simulator that, based on the previous characterization, simulates the transmission of a DNA-packet between two nano-machines.

Keywords: Nano-networks; Molecular Communication; Flagellated Bacteria; DNA Packet; Propagation delay

[*]Corresponding author. Tel.: +34 934054063; fax: +34 934017055.

[**]While staying at Broadband Wireless Networking (BWN) Laboratory, School of Electrical and Computer Engineering, Georgia Institute of Technology, 250 14th Street, Atlanta, GA 30332, USA

Email addresses: mgregori@ac.upc.edu (Maria Gregori), llatser@ac.upc.edu (Ignacio Llatser), acabello@ac.upc.edu (Albert Cabellos-Aparicio), ealarcon@eel.upc.edu (Eduard Alarcón)

1. Introduction

Nanotechnology is a multidisciplinary field based on knowledge of diverse scientific areas and with potential applications in several fields such as biomedical, environmental or industrial (e.g., [1]). A nano-machine is the most basic functional unit able to perform very simple tasks at the nano-scale. These tasks include computing, data storage, sensing and actuation among others [2].

Currently several approaches have been proposed to build nano-machines. First, the top-down approach is based on downscaling current micro-electronic and mechanical devices to the nano-scale [3, 4]. This promising approach is still in an early stage. Second, in the bottom-up approach, nano-machines are assembled based on individual building blocks [5]. Current synthetic chemistry technology is able to prepare small molecules to almost any structure, and it is envisioned that in the future these techniques may be used to develop nano-machines by arranging molecule by molecule [26]. This process is called molecular manufacturing and may be developed from current technologies in a decade if adequate research efforts are devoted. And finally, in the bio-inspired approach, nano-machines can be built by taking advantage of existing biological nano-machines (e.g, cell receptors). This approach proposes to use these biological nano-machines either as models to develop new machines, or as building blocks. This technique offers promising solutions in the short term.

In this scenario, nano-networks [2] are the inter-connection of nano-machines, and as such expand the capabilities of a single nano-machine. Nano-networks can provide means for cooperation and information sharing among nano-machines, allowing them to fulfill more complex tasks.

Several techniques have been proposed to interconnect nano-machines [2]. Among the most promising ones is molecular communications, which is based on the use of molecules to encode the desired information and transmit it by mimicking biological systems found in nature. As a consequence, this communication paradigm is particularly suited to bio-inspired nano-machines.

As it happens in nature, molecular communication should be tackled in different ways depending on the distance between emitters and receivers. Two different approaches have been already proposed for short and long-range communications.

For short-range communications (nm-μm), researchers are proposing to use either molecular signaling [27] or molecular motors [28]. The former

method encodes information in variations of the concentration of the molecules while the latter technique encodes the information into the molecules themselves. Concerning long-range communications (mm-m), pheromones [29] have been proposed to carry information. As seen in nature, pheromones emitted by a member of certain species can only be detected by other members of the same species. Similarly, pheromones transmitted by a particular type of nano-machines may only be detected by other nano-machines equipped with the corresponding decoder.

The existing short-range molecular communication methods do not seem to be effective for distances longer than a few μm, but still, nano-machines should be able to communicate with other nano-machines irrespectively of their distances. For instance, molecular communication techniques based on diffusion (such as calcium signaling or pheromones) show a very high attenuation and delay for distances above a few tens of μm [22]. Furthermore, molecular motors are not appropriate for the medium range due to their low propagation speed, in the order of 500 nm/s [32], among other reasons. For this reason, in a previous work [6], we introduced two mechanisms intended for medium-range communications (μm to mm) along with a nano-network architecture. In particular we introduced flagellated bacteria and catalytic nanomotors. Both methods are based on encoding information in DNA sequences (a *DNA packet*) and carrying it to the receiver. Our proposed network architecture assumes clusters of nano-machines that communicate among them using short-range mechanisms, and gateways that, taking advantage of medium-range techniques, inter-connect clusters.

Following the nanonetwork architecture introduced in [6], the communication between two nanomachines is as follows (Fig. 1): first, the transmitter nano-machine transmits the desired information to its gateway by using short-range techniques, i.e. the information could be a DNA string and transported to the gateway by means of molecular motors. The gateway node, which is envisioned as a DNA computer, multiplexes short-range packets of different nano-machines that have the same destination gateway, hence, creating the medium-range packet (more details in the proposed multiplexing scheme are presented in [6]). This packet is then encoded inside a flagellated bacterium that transports the information to the receiver's gateway. Then, the receiver's gateway demultiplexes the medium-range packet into different short-range packets and relays these packets to the corresponding receiver nano-machines by means of short-range techniques.

In this paper, we model the communication between gateway nodes by
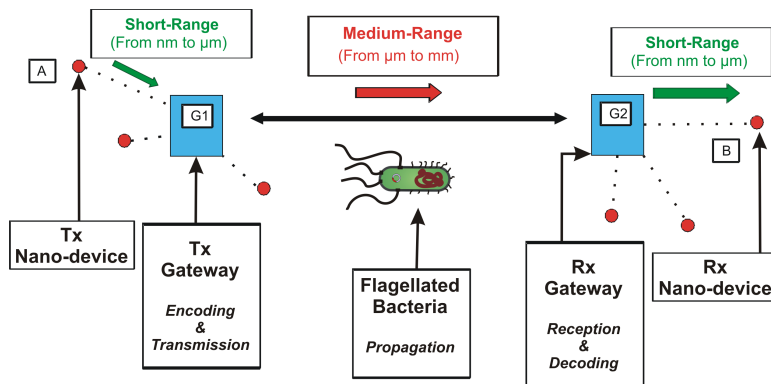
Figure 1: Communication process using flagellated bacteria

presenting a physical channel characterization for flagellated bacteria. A scheme of the communication proccess is shown in figure 1. First, we describe the proposed mechanism to encode, transmit and decode information using flagellated bacteria. Secondly, we characterize the random walk followed by the bacterium in order to propagate the information. As we will see, this random walk depends on the concentration of attractants. Finally we have developed a simulator which allow us to quantify the propagation delay and the packet loss probability. Researchers working in nano-network architectures may consider these results when designing and/or evaluating novel paradigms.

2. Background: Flagellated Bacteria

2.1. Overview

Bacteria have spent several billion years developing skills and efficient machinery, as cilia and flagellum, that allow them to convert chemical energy into motion. For instance, Escherichia coli (*E. coli*), which is shown in figure 2, has between 4 and 10 flagella moved by rotary motors located at the cell membrane and fuelled by chemical compounds. *E. coli* also has several pili distributed around its outer membrane that give the bacterium the ability to cohere other cells in order to exchange genetic material, which is carried out by a cellular process called bacterial conjugation.

Among all possible flagellated bacteria we focus on *E. coli* because it is the most studied prokaryotic cell, and its complete genome sequence is well-known [7]. *E. coli* is approximately 2 μm long and has a diameter of
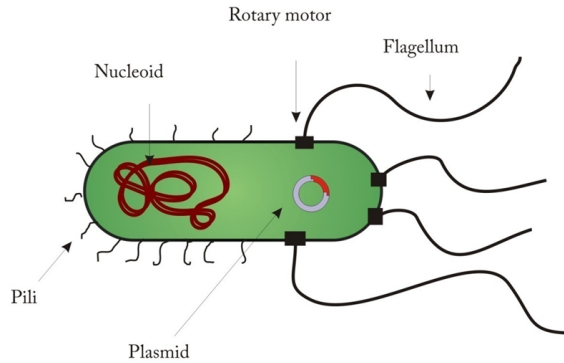
4

Figure 2: Flagellated Bacteria

1 μm, and it is usually an inoffensive bacterium that lives in the human intestinal tract. Its nucleoid contains only one circular DNA molecule and in its cytoplasm there are some smaller DNA sequences (called plasmids [8]) arranged in a circular way. Plasmids can give the bacteria resistance to some antibiotics in the environment, but they are also used in genetic engineering to conduct genetic manipulation experiments [9].

In this work, we propose to use flagellated bacteria to carry DNA messages to the appropiate receivers. In particular we propose to choose a specific mutant of the bacteria that only responds to a subset of attractants [11]. The proposed communication scheme is as follows: first, the DNA message is introduced inside the bacterium cytoplasm. Then, the bacterium is released into the environment that in turn will follow its natural instincts and will propel itself to a particular receiver, which is continuously releasing attractant particles to the environment. Please note that this mechanism differs from the traditional ones since since the receier has to actively release attractants in order to receive information. Finally, in the last step, the reception and decoding of the DNA message is done by exchanging the genetic material between the bacterium and the final destination.

Flagellated bacteria requires that the receivers (gateways), mimicking the design of certain natural cells, contain a reservoir of attractants and mechanisms to release them. Clearly, there is an analogy between energy in todays networks and the attractants in nano-networks, since both limit the capabilities of these systems. In order to refill the gatewayss reservoir, that is re-charge them (following the energy analogy), several mechanisms can be used. For instance a micro-device could release large quantities of attractants

in the environment that in turn could be captured by the gateways. This should be done at a certain time frames in order to avoid disrupting the existing communications.

In the following subsections we describe the communication process in detail:

2.2. Encoding & Transmission

Instead of working with the common binary alphabet as today's computers, some researchers argue that nano-machines will be able to work with a quaternary alphabet composed by the DNA nucleotides: Adenine, Thymine, Cytosine and Guanine (A, T, C and G) [12]. In this scenario, the information that the emitter nano-machine transmits is expressed as a set of DNA base pairs (i.e., the DNA packet).

Encoding is the process by which the DNA packet is inserted inside the bacteria's cytoplasm. This insertion can be done using a set of techniques: plasmids, bacteriophages or Bacterial Artificial Chromosomes. All of them are well-known and widely used in fields like biology or pharmacy.

Firstly, plasmids are circular sequences of DNA [13], with length between 5000 and 400000 base pairs (equivalent to 10 to 800 kbits), that can be inserted inside bacterial cells by transformation or electroporation [8]. Secondly, bacteriophages are a type of viruses, smaller than bacteria, able to infect bacteria with its genetic material. And third, Bacterial Artificial Chromosomes (BAC) are artificial plasmids designed for cloning long segments, up to 300000 base pairs of DNA (equivalent to 600 kbits). The procedure used to encode the message inside the BAC is the same than the one used for plasmids. It is worth to note that in this case the host bacteria must be genetically modified in order to allow the entrance of the long BAC vector through the membrane.

The maximum packet size depends on the method used for encoding the information inside bacteria. It was reported in [8] that using plasmids it is difficult to clone (in our case, encode) sequences longer than 15000 base pairs, whereas the bacteriophages mechanism enables cloning of 23000 base pairs. The most effective method is BAC, which enables to encode up to 300000 base pairs.

Unfortunately, all these methods have not yet been performed without external intervention from the macro-scale. For this reason, we propose the following approach. In early implementations, *E. coli* libraries could be created, where each *E. coli* will have different pre-established encoded informa-

tion, so different DNA packets. These bacteria could be stored in the gateway node, in a kind of warehouse, and each bacterium will be resistant to a specific antibiotic which will allow the selection of the correct bacterium. By applying the antibiotic to a small group of bacteria, the gateway can select and release the desired bacterium, which contains the desired DNA information, to the medium when it is necessary (the other bacteria will die by the effect of the antibiotic). Since *E. coli*, as all bacteria, are able to reproduce, so create a new bacteria with the same genome, new bacteria are constantly created, this ensures that the warehouse will never be depleted. It is important to remark that selecting the bacterium by using antibiotics and having pre-established encoding information, will simplify the design of the gateway node in early implementations, however, it will also limit the capacity of the system. Hence, more research is required on how to implement the encoding schemes inside the gateway node.

There are several issues that must be further studied:

- How the alien piece of DNA affects the bacterium, in other words, it must be controlled that this piece of foreign DNA will not harm the carrier bacterium nor create a strand of harmful bacteria. These could be avoided by deleting the origin of replication of the plasmid. However, if the plasmid is not allowed to be cloned, the redundancy of the message is lost, which is an important feature for obtaining robustness of the message in a molecular communication network. Other alternatives must also be studied, such as encoding the information as proteins coated with vesicles.

- The encoding technique to be used must be determined by taking into account both the integration feasibility in the micro-scale, i.e., in the gateway node, and the requirements of the amount of information to be transmitted. Indeed, nanotechnology offers new solutions which can lead to rethinking of the current techniques. For instance, the plasmid could be synthesized from scratch in the gateway node by using DNA computing operations, and inserted inside the bacterium by using an artificial bio-inspired pilus, hence mimicking the bacterial conjugation process.

- The self-reproduction ability of bacteria carries several advantages, but also a few drawbacks. The most obvious advantage is that bacteria

7

self-reproduction naturally generates redundancy in the message. Redundancy offers several benefits, such as a lower packet loss probability and a lower mean propagation time. However, self-reproduction also has some drawbacks, including the overpopulation of bacteria in the environment and the introduction of errors in the message due to mutations [33].

2.3. Propagation

Bacteria have a great number of chemical receptors surrounding its membrane that allow them to sense the environment for the presence of attractant particles and move towards them, this process is called chemotaxis. Bacterial chemotaxis is a nature marvel example of signal transduction and it has been widely studied in the literature [14, 15].

E. coli moves in series of "runs" and "tumbles" [16]. In each run, the flagella motors spin counterclockwise, and the bacterium swims roughly in straight line. A tumble is a small period of time during which the bacterium moves erratically because several filaments are spinning clockwise. During a running period, bacteria sense the amount of nutrients (sugars, amino acids and dipeptides) in the environment several times by using its cell membrane's chemoreceptors [17]. By comparing the obtained results, the bacterium is able to decide whether the nutrient concentration is increasing or decreasing. If the concentration is increasing, the running time will be longer, so the rotary motor spins counterclockwise during a longer period of time. This bias in the running time enables cells to find the places where the environment is better (in terms of nutrients).

In recent decades, an exhaustive research has been conducted in understanding how the flagellar motor of bacteria works. Its structure, parts, and how these parts are assembled are well known [31]. Information regarding the fuel that it uses, the torque that it can generate at different speeds and what controls the likelihood of the direction changes are also well documented in the literature. However, it is still unknown what makes the bacterium run or tumble and what makes the motor change from one state to another. For this reason, bacterial mobility still has a small random component that is being widely studied [20, 15, 23]. This random component must be modeled in order to find out the time required for the bacteria to move from the emitter to the receiver. In sections 3 and 4, we elaborate on how the environment affects the bacteria movement, and we model the bacterial movement, respectively. In section 5, we describe a developed simulation tool which characterizes how

bacteria move in a point to point communication and estimates the required propagation time.

2.4. Reception & Decoding

The reception of a DNA packet can be done following a natural cellular process called Bacterial Conjugation that is defined as the exchange of genetic material among bacteria cells [13]. The exchange of these circular sequences of genetic material (plasmids) is possible through direct contact between cells. This contact is achieved by means of the bacterial appendage called pilus, and the process is as follows. The donor bacterium localizes the receiver cell, which in our case is the receiver's node, and attaches to it using the pilus. Then the bacterium retracts the pilus in order to have direct contact with the receiver. This contact makes both membranes to fuse together, in a kind of bridge by which the donor bacterium transfers a single strand of the plasmid DNA. When both donor and recipient cells have a single strand of the plasmid, DNA synthesis must be done (by both of them) in order to recover the whole plasmid.

Once the plasmid is at the receiver, the DNA packet must be extracted from the plasmid. This is done by restriction endonucleases enzymes that cleave the plasmid in restriction sites. When the plasmid has been cleaved, the receiver nano-machine is able to use and process the DNA packet.

3. Environment

In this section we describe the environment in which the bacterium lives and transmits the information. This is required as a first step to characterize the physical channel model. First, we describe the mechanism used to propel the bacteria and the forces affecting it at the micro-scale. Secondly, we focus on the diffusion of attractants, whose concentration is sensed by the bacteria.

3.1. Bacteria Propulsion in Low Reynolds Numbers

The Reynolds number [18] is a dimensionless number that expresses a ratio between inertial and viscous forces of a given object:

$$R = \frac{\text{Initial forces}}{\text{Viscous forces}} \tag{1}$$

Bacteria movement is governed by a set of forces completely different from the forces that govern motion of objects at the macro scale. From the *E. coli*

bacteria point of view, water is a granulated substance through which it has to swim, and hence, bacterial movement is not constrained by inertial forces but by viscous forces. For this reason it is considered that bacteria move in low Reynolds numbers [19].

For a swimming organism, the Reynolds Number is given by:

$$R = \frac{lv\rho}{\eta} \tag{2}$$

where l is the size of the organism, v its velocity and, ρ and η are the density and viscosity of the medium respectively [19].

For instance, an *E. coli* bacterium swimming fast in water has a Reynolds coefficient of $R = 10^{-5}$, whereas, a human paddling in a pool experiences a Reynolds coefficient of $R = 10^5$. In order to understand the forces acting upon a swimming bacterium, it was proposed in [19] to imagine yourself trying to move in a swimming pool full of molasses, and that you are not allowed to move the parts of your body faster than the hands of a clock.

Natural selection has driven microorganisms to develop efficient machinery and tools that allow them to propel themselves in low Reynolds numbers environments. *E. coli* bacteria have developed complex rotary motors that are powered by protons or sodium ions (Na^+) flux [20]. These motors are able to produce a rotary movement of the flagellum either in a clockwise or counterclockwise way.

When an *E. coli* is swimming at constant velocity, the rotary motors spin counterclockwise and the cell body spins clockwise, as shown in figure 3. Since the velocity is constant, the net force experienced by the bacterium must be zero. Otherwise, it would either accelerate or decelerate. Taking this into consideration, the torque generated by rotation of the filaments is balanced by viscous drag due to counter-rotation of the body of the cell, and thrust generated by rotation of the filaments is balanced by viscous drag due to translation of the body of the cell.
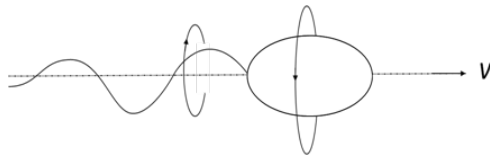

Figure 3: Bacterium swimming at constant velocity v

3.2. Diffusion of Attractants

In the proposed model the receiver node is constantly releasing attractant particles to the environment. These particles diffuse through the medium and generate concentration gradients, which are sensed and followed by bacteria. In order to quantify the time required for a bacterium to reach the receiver, first we must model the diffusion of the attractants through the environment.

When in a certain environment there exists a non-uniform distribution of particles, these tend to diffuse away in order to reach a uniform concentration through all the space [21]. The flux of particles is obtained using Fick's first equation (eq. 3), which states that the net flux of particles in a certain position and time is equal to the spatial gradient of the particle concentration $c(\bar{x}, t)$ multiplied by the diffusion coefficient D.

$$\bar{J}(\bar{x}, t) = -D\bar{\nabla}c(\bar{x}, t) \tag{3}$$

where $\bar{\nabla}c(\bar{x}, t) = (\frac{\delta c(\bar{x}, t)}{\delta x_1} \frac{\delta c(\bar{x}, t)}{\delta x_2} \cdots \frac{\delta c(\bar{x}, t)}{\delta x_n})$ is a vector that has the same dimension than $\bar{x}$ and $\bar{J}$.

The diffusion coefficient D for spherical particles moving in low Reynolds number fluids is expressed as follows [19]:

$$D = \frac{K_b T}{6\pi\eta r} \tag{4}$$

where r is the radius of the particle, η and T are the viscosity and temperature of the medium respectively and K_b is the Boltzmann constant.

The continuity equation (eq. 5) states that particles cannot be created or destroyed; thus, the number of particles entering and leaving the system must coincide. The principle states that the time derivative of the particle concentration $\frac{\delta c(\bar{x}, t)}{\delta t}$ at location $\bar{x}$ and time t is equal to the opposite of the particle concentration flux $\bar{J}(\bar{x}, t)$ at location $\bar{x}$ and time t.

$$\frac{\delta c(\bar{x}, t)}{\delta t} = -\nabla\bar{J}(\bar{x}, t) \tag{5}$$

The second Fick's law (eq. 6) is obtained by substituting the first Fick's law (eq. 3) into the continuity principle (eq. 5):

$$\frac{\delta c(\bar{x}, t)}{\delta t} = D\nabla^2 c(\bar{x}, t) \tag{6}$$

11

where ∇^2 is the Laplace operator which expresses the divergence of the gradient, hence $\nabla^2 c(\bar{x}, t) = \frac{\delta^2 c(\bar{x},t)}{\delta^2 x_1} + \frac{\delta^2 c(\bar{x},t)}{\delta^2 x_2} + \cdots + \frac{\delta^2 c(\bar{x},t)}{\delta^2 x_n}$

As stated before, the receiver is constantly adding particles into the environment and the concentration is constantly increasing, hence the continuity principle is not satisfied. Under this constraint, we are not allowed to use the second Fick's equation (eq. 6).

In order to satisfy the continuity principle, we sample the concentration in the environment in small periods of time, Δt. If Δt is small enough, we can assume that, in this time interval, the number of particles in the system is constant, and therefore the continuity principle is fulfilled.

Hence, we can express the second Fick's Law using the finite differences method in a discrete environment as:

$$\frac{c(\bar{x}, t + \Delta t) - c(\bar{x}, t)}{\Delta t} = D \frac{c(\bar{x} - \Delta\bar{x}, t) - 2c(\bar{x}, t) + c(\bar{x} + \Delta\bar{x}, t)}{(\Delta\bar{x})^2} \quad (7)$$

In order to use the finite differences scheme we must verify that the system is stable. This is verified when time interval Δt, is smaller than the length interval, $\Delta\bar{x}$, divided by two times the diffusion coefficient, that is:

$$\Delta t \leq \frac{(\Delta\bar{x})^2}{2D} \quad (8)$$

4. Random Walk

Recently, exhaustive research has been conducted in order to understand how the flagellar motor of bacteria works. Research on this topic is mostly experimental, and although there is no closed expression for the runs and tumbles of bacteria, empirical observations have shown that bacteria's movements have a random component. In this section we model the biased random walk that describes the movements of the bacteria along with its response to the concentration of attractants.

4.1. Overview

When an attractant particle binds to one of the chemoreceptors placed at the cell membrane of the bacterium, it triggers a complex pathway of chemical signals that regulates the motion of flagella's rotaries motors. The interested reader can find more information about this process in [20]. In

this subsection we aim to model it. Our starting point is [15], where the authors developed a microscope able to track and analyze the movement of bacteria in a three dimensional environment. The researchers carried out two experiments. In the first one, they introduced the bacteria in a homogenous environment, where the concentration was uniform. In the second experiment they introduced gradients of attractant particles using capillary tubes.

The first experiment revealed that the bacterium moves in a series of running and tumbling periods. They observed that during a running period, the cell swims at a constant velocity in an almost unidirectional direction. However, the cell drifts due to the rotational diffusion which produces small changes in its direction, as a result, the cell meanders during a run. In a tumble period the bacterium slows down or stops and changes its direction with an angle γ. Specifically they observed that the probability density function of γ was not uniform, but small changes on direction where more likely. Moreover, they repeated the experiment several times and they discovered that the run length and tumble length times are exponentially distributed.

In the second experiment they observed clouds of bacteria close to the mouths of the capillaries sank. By tracking run and tumbling lengths the authors were able to understand how bacteria move towards places with higher concentrations. In this experiment they observed the same behavior in terms of changes in direction and run and tumble length time distributions. The main difference is that the mean run length increases when the bacterium moves up the gradient, whereas when it is moving down gradients the mean run length is similar than that observed in homogeneous solutions. This increase in the mean run length results in a random walk model with a preferred direction, that is, a *biased random walk model.*

In the following subsections we describe the model and the parameters that govern the movement of bacteria, namely rotational diffusion, run and tumbling lengths and changes in direction.

4.2. Changes in Direction due to Rotational Diffusion

As explained in the previous section, the forces required by bacteria to move in water are governed by low Reynolds numbers. From the *E. coli* perspective, water looks as a set of granulated particles that are continuously moving. When the bacterium wants to swim, it has to drag these particles causing the fluid to shear. Collisions with water molecules prevent the cell from moving in a straight line; as a result, the bacterium drifts. This drift is

commonly known as rotational diffusion and drives the cell to deviate more than 60 degrees in 10 seconds [15].

In this section, we aim to characterize the rotational diffusion, which can be analyzed as a rotational random walk [24] where every τ seconds the bacterium rotates an angle of $\pm\phi$ degrees. We define the random variable $\theta_j(n)$ as the angle the bacterium j has rotated after a certain time $t = n\tau$, and we aim to characterize its mean and variance. With this purpose, we observe that, for large values of t and assuming that each angular displacement is independent from the previous ones, $\theta_j(n)$ follows a Brownian motion model. Thus, the angular displacement can be modeled as a Gaussian process with mean $E[\theta(t)] = 0$ and variance given by [30]:

$$var[\theta(t)] = 2D_r t \tag{9}$$

where D_r is the rotational diffusion coefficient [24] and t is the elapsed time. We can express the rotational diffusion coefficient D_r as a function of the rotational frictional drag coefficient f_r, given by the Einstein-Smoluchowski relation:

$$D_r = \frac{K_b T}{f_r} \tag{10}$$

where K_b is the Boltzmann constant and T is the temperature of the environment. Assuming that the bacterium is modeled as a sphere of radius a, and that it is placed in an environment with viscosity η, its rotational frictional drag coefficient can be expressed as $f_r = 8\pi\eta a3$. Then, we can derive the rotational diffusion coefficient as:

$$D_r = \frac{K_b T}{8\pi\eta a3} \tag{11}$$

For example, if we assume that the bacterium radius is $a = 1 \ \mu m$, and that the environment has a temperature $T = 305 \ K$ and a viscosity $\eta = 0.027 \ g/(cms)$, we obtain $D_r = 0.062 \ rad2/s$. Figure 4 shows the probability density function of the angular deviation of the bacterium after 10 seconds in a running period, which will be a normally distributed random variable with mean $E[\theta(n)] = 0$ and variance $var[\theta(t)] = 1.12 \ rad^2 = 64.2$ degrees^2.

4.3. Changes in Direction in Tumbling Periods

Having characterized the drift of the movement of bacteria, in this section we focus on their changes in direction. Between two consecutives runs
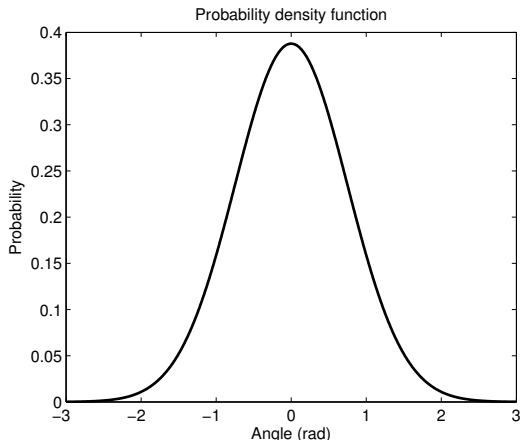
Figure 4: Probability density function of the angular deviation

bacteria tumble, sense the environment for attractants and choose a new direction. We define the random variables θ_n and θ_{n+1} as the angles that determine the bacterium direction in two consecutives runs. The angle θ_{n+1} can be obtained as a function of the previous angle, θ_n and a random angle γ:

$$\theta_{n+1} = \theta_n + \gamma \tag{12}$$

In order to characterize γ, we rely on the empirical measurements carried out in [15]. In particular, the authors measured the probability distribution of γ between two runs, shown in figure 5. Based on the authors' measurements, we approximate the probability density function $f(\gamma)$ as:

$$f(\gamma) = \begin{cases} \frac{1}{4}\cos(\gamma/2) & |\gamma| \leq \pi \\ 0 & |\gamma| > \pi \end{cases} \tag{13}$$

Hence, in the simulation the angle of each running period, θ_{n+1} is computed as the angle of the previous run θ_n plus a random number γ generated following the distribution given by eq. 13 and shown in figure 6.

4.4. Run and Tumble Lengths

In this section we focus on run and tumble lengths of bacteria. One may wonder why E. coli tumbles; the main reason is that E. coli is aware of the physics of the medium where it is living. The bacterium knows that after
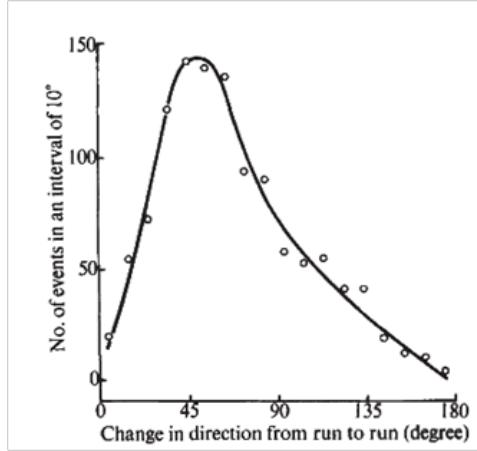
15

Figure 5: Distribution of changes in direction between runs (extracted from [15])

ten seconds swimming in straight line, the rotational diffusion will cause a deviation on its initial course of more than 60 degrees on average. At this point *E. coli* stops, senses the environment and takes another direction. Therefore, the upper limit of the running time is set by the effect of the rotational diffusion.

The lower limit is fixed by the time required by the bacterium to sense, count and process the information regarding the concentration of attractants and repellents in the environment. Since attractants move according to Brownian motion, their expected displacement in a time t is equal to $\sqrt{2Dt}$, where D is the medium diffusion coeficient. Thus, if the bacterium remains still in one place for a period of time t, it will sense in average the attractant particles that are diffusing from a distance at most $\sqrt{2Dt}$. When the bacterium is swimming with constant velocity v, if it wants to verify whether the attractants concentration is increasing or decreasing, it must outrun the diffusion of particles. This implies that $vt > \sqrt{2Dt}$. As a result, a rough approximation of the minimum time required for the bacteria to sense the concentration is:

$$t_{min} = \frac{2D}{v^2} \tag{14}$$

The diffusion coefficient D depends on the specific attractants sensed by bacteria. Bacteria have a large set of chemoreceptors in its membrane. Each kind of chemoreceptor reacts with a different attractant and presents different efficiencies and sensibility [17]. Aspartate and Serine attractants present the
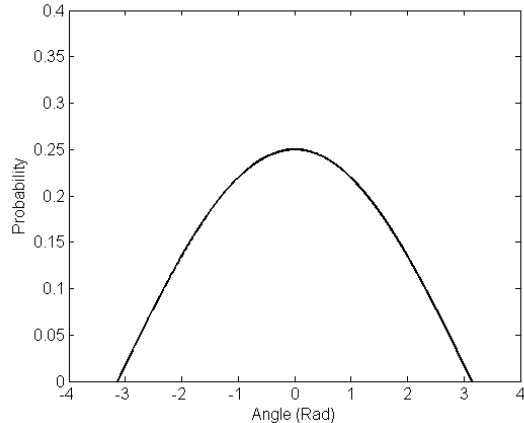
16

Figure 6: Probability density function of γ

best response and precision; for this reason, we assume these attractants in our model ($D = 10^{-9}m^2/s$) [20]. Other attractants could be chosen as well, provided that they are chemically compatible and thus their interaction does not trigger a chemical reaction. Hence, given D, if we assume that the bacterium is moving with a velocity of $v = 30$ μm/s, the minimum run time is $t_{min} \approx 1$ s.

Run Length: We consider an event as a change in the bacterium state from run to tumble. Since it has been empirically observed that both the running and tumbling lengths are exponentially distributed [15], we can model the event "the bacterium state changes from run to tumble" as an exponential random variable. Then, we can obtain the probability that this event occurs in a certain instant t_n as:

$$P(t_n; \lambda) = \lambda e^{-\lambda t_n} \qquad (15)$$

where λ is the mean run length of the bacterium.

Tumbling Length: Similarly, the probability of the event "the bacterium state changes from tumble to run" occuring in a given time t_n is:

$$P(t_n; \mu) = \mu e^{-\mu t_n} \qquad (16)$$

where μ is the mean tumble length of the bacterium. In this case, $\mu = 0.1$ seconds [20].

17

Tumbling Rate: The tumbling rate $\alpha(t)$ is the inverse of the mean run length $\lambda(t)$, so it can be expressed as:

$$\alpha(t) = \frac{1}{\lambda(t)} \tag{17}$$

We represent here λ as a function of time, since it has been found that the mean run length depends on whether the bacterium is going in the right or wrong direction, that is, ascending or descending the gradient of attractant particles. If the bacterium is going down the gradient, the mean run length is constant and we can approximate it as $\lambda = 1$ second; whereas if it is going up the gradient, the mean run length is longer and we can obtain it as a function of the sensed concentration $c(t)$ [20]. The process by which the bacterium obtains this function is explained in section 4.5.

4.5. Impulse Responses in Bacterial Chemotaxis

The behavior of the bacteria when exposed to brief impulses of attractants has been previously measured in [25]. In order to obtain the bacterium's response to an attractant impulse, a flagellum of the cell is tethered to a glass surface and the response of the rotary motor is monitored, measuring the probability that the motor spins counterclockwise, hence, the probability that the cell is running.

The top plot in figure 7 shows the probability that the bacteria starts running when a stimulus of an attractant is applied, whereas the bottom plot corresponds to the case when a repellent stimulus is applied to the cell. Both stimuli are applied at the time instant $t = 5$ s. When the bacterium senses an attractant stimulus, its response appears after 0.2 seconds and it has a peak 0.4 seconds after applying the stimulus. Then, the probability drops again, crossing the baseline at the time instant $t = 6$ s. The response reaches a minimum at $t = 6.5$ s, and finally it goes back to its initial value about 4 seconds after the application of the stimulus.

In light of these empirical results, we assume that the run probability in front of the attractant concentration satisfies the properties of *linearity* and *time-invariance*. Then, we can model the run probability of bacteria as a system characterized by an impulse-response $h(t)$ and whose input is the concentration sensed by the bacterium $c(t)$. Note that the whole communication system may not be linear and/or time-invariant, only the run probability is modeled as a linear invariant system. Based on the empirical observations from [25], we model the impulse response of the run probability
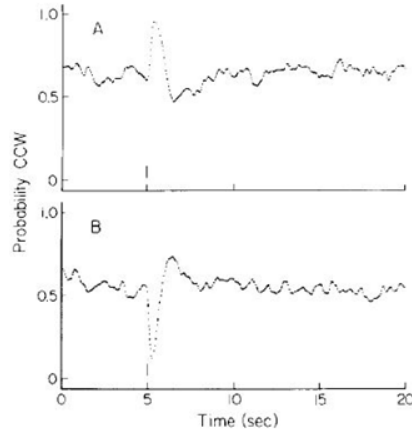
Figure 7: Temporal evolution of the run probability of *E. coli* bacteria when exposed to attractants (above) and repellents (below) (extracted from [25])

of the bacterium using spline interpolation; we obtain the function shown in figure 8.

The Fourier transform of the impulse response is represented in figure 9, which shows that bacteria behave as a low pass filter of the concentration in the environment with a cut-off frequency of $f_c = 0.64$ Hz.
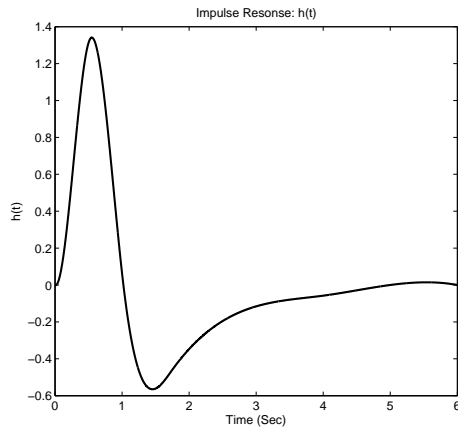

Figure 8: Normalized impulse response of the run probability of flagellated bacteria

The output of this system is the convolution of the sensed concentration and the impulse response. This output $y(t)$ is used by the bacterium to
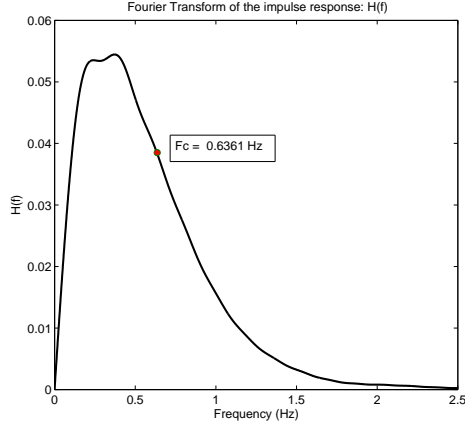
19

Figure 9: Fourier Transform of the impulse-response

determine the mean run length $\lambda(t)$, or its inverse, the tumbling rate $\alpha(t)$. The tumbling rate can be obtained as follows:

$$\alpha(t) = \frac{1}{\lambda(t)} = \begin{cases} 1 - ky(t) & y(t) > 0 \\ 1 & y(t) \leq 0 \end{cases} \qquad (18)$$

where $y(t) = \int_0^\infty h(t)c(t - \tau)d\tau$ and k is a constant that normalizes its energy.

5. Simulation Tool

In this section we describe the simulation tool that we have developed to evaluate a point-to-point link using a flagellated bacterium as information carrier. First we describe the model used to simulate the diffusion of particle attractants and then the simulation model for the bacterium itself.

5.1. Simulation of Attractant Particle Diffusion

Recalling section 3, using eq. 7 and choosing the parameter correctly to satisfy eq. 8, we can simulate how the attractant particles that the receiver is releasing diffuse through the environment. That is, the concentration that a bacterium senses when moving towards the receiver. The parameters used in our simulation are the following:

20

Diffusion Coefficient: As stated before, we assume that the specific attractants used by the receiver are Aspartate and Serine. Their diffusion coefficient in water is $D = 10^{-9} m^2/s$ [20].

Time and Length Intervals: The time interval, Δt, and the length interval, $\Delta \bar{x}$, have been chosen in order to satisfy the stability eq. 8. We have also taken into account that Δt must be small enough to let the bacteria have enough resolution of attractant concentration. Hence, the values used in our simulation are $\Delta t = 10^{-2}$ s and $\Delta \bar{x} = 10^{-5}$ m.

The receiver nano-machine, which is constantly releasing attractant particles, is placed in the middle of the simulated space. We assume that the concentration gradient becomes stable after a certain time. The results presented in figure 10 have been taken after 10 minutes of simulation and it is easy to observe that the diffusion is an isotropic process. Moreover, the concentration gradient increases when approaching to the source of attractants, the receiver nano-machine. This increase in the concentration gradient is sensed by bacterium's chemoreceptors, which leads the organism to regions with high concentration of attractants.
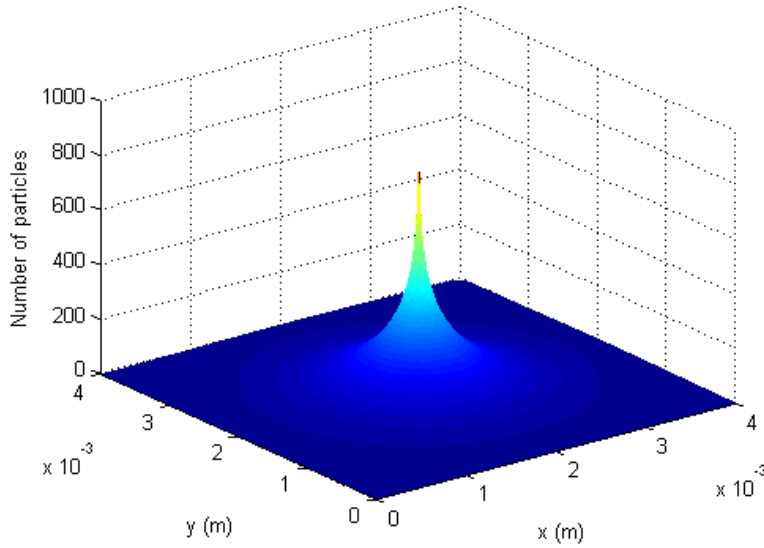

Figure 10: Diffusion of attractant particles

5.2. Simulation of the Movement of Flagellated Bacteria

In this subsection we describe the simulation tool described to simulate the bacterium. This simulation tool takes into account all the parameters that define bacteria behavior, which have been introduced in the previous sections. With this model, we are not only able to obtain the path that a certain bacterium follows, but also the required time to reach the receiver, i.e., the propagation time. In case that the bacterium does not reach the receiver after a certain time t_{max}, we assume that the bacterium is lost, and hence, that the transmitted packet is lost.

The movements carried by the simulated bacterium depend on the present state. After reading the concentration on the environment, the bacterium computes the probability to change the state and decides either to change or remain in the same state. These probabilities are computed by taking into account the mean run and tumbling lengths of the bacterium (see section 4.4). When the bacterium is running, it moves 0.2 μm in the direction given by θ, otherwise it remains in the same position. The rotational diffusion, see section 4.2, is simulated by generating a random number with the variance given by eq. 9. The changes in direction between runs have been obtained by generating a random number distributed following the probability density function shown in figure 6 (see section 4.3).

The details about the parameters used in the simulators along with its pseudo-code can be found in the appendix.

6. Simulation Results

This section presents the simulation results. We focus first on figure 11, which shows the trace of a single bacterium from the transmitter (square) to the receiver (circle). We observe that the bacterium moves by following a biased random walk model, as discussed in section 4. We can observe that, when the bacterium is going towards the receiver, the runs are longer than when it is heading in the opposite direction. Moreover, the plot shows that, when the bacterium is running, its direction is slightly affected by the rotational diffusion; whereas its changes of direction are larger when it tumbles.

The results in terms of the propagation time of the bacteria from the transmitter to the receiver are shown in figure 12. We carried out 1000 simulations using different distances, ranging from 50 μm to 1 mm, in steps of 50 μm. We define $t_{prop}(d)$ as a random variable that accounts for the
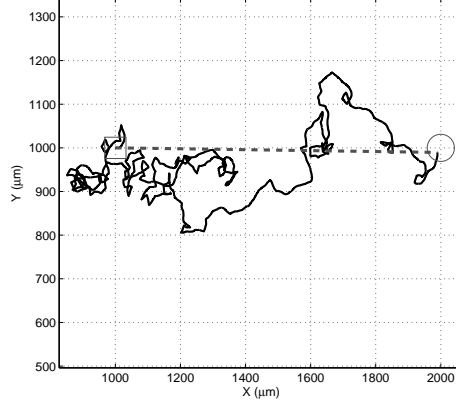
Figure 11: Trace of the bacterium from the transmitter (square) to the receiver (circle)

propagation time of the bacteria for a given distance between transmitter and receiver d. By averaging the results, we obtain the mean propagation time $E[t_{prop}(d)]$ in minutes as a function of the distance, shown in figure 12. The squares correspond to the times obtained through simulation, and the solid line is the approximation of the propagation time $\bar{t}_{prop}(d)$ by a second order polynomial, obtained by polynomial fitting:

$$\bar{t}_{prop}(d) = 1.82d^2 + 4.49d + 0.17 \tag{19}$$

This second order polynomial $\bar{t}_{prop}$ effectively approximates the mean propagation time t_{prop} in minutes as a function of the distance d in mm.

Moreover, for a given distance it is possible to analyze how the arrivals are distributed as a function of time. In figures 13(a) and 13(b), the dots represent a histogram of the number of bacteria arrivals as a function of time, for distances of $150\mu m$ and $1000\mu m$, respectively. The probability distribution of the propagation time can be approximated with a gamma distribution, which represents the sum of k independent exponentially distributed random variables, each of which has a mean of θ. The gamma distribution has the following probability density function:

$$f(x, k, \theta) = x^{k-1} \frac{e^{-x/\theta}}{\theta^k \Gamma(k)} \qquad \text{for } x, k, \theta > 0 \tag{20}$$

where Γ represents the gamma function. The choice of this model is
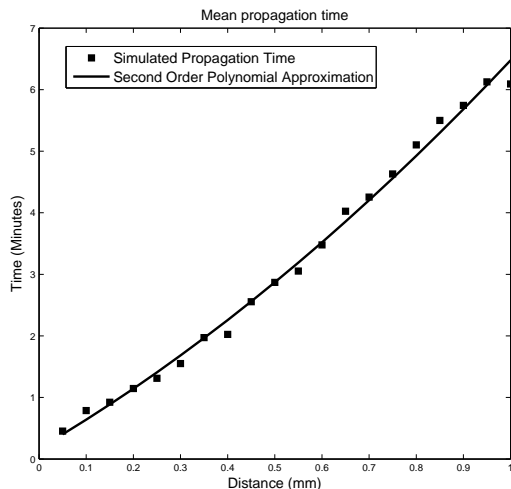
23

Figure 12: Mean Propagation Time of flagellated bacteria

motivated by the actual movement of bacteria: since the run lengths are exponentially distributed and approximately independent, the gamma distribution is expected to be a good fit for the propagation time. The mean propagation time of bacteria for a given distance can then be expressed as $E[t_{prop}(d)] = k\theta$, where k is the average number of runs and θ the mean run length.

This approximation is obtained by curve fitting and shown as a continuous line in figures 13(a) and 13(b). The adjusted R-square values are 0.96 and 0.89, respectively, confirming the goodness of the fits.

It is worth to note that bacteria contain a large number of chemicals receptors surrounding its membrance used to sense the attractants. These sensors have a natural limit on its sensitivity and cannot sense the attractants at certain low concentrations. Thus, the maximum distance that a bacterium can travel depends on the concentration of attractants released by the receptor, and the minimum concentration that the chemoreceptors are able to sense. We refer the reader to [14] for more details about this.

Concerning packet losses, in our simulations all the bacteria reached their destination. However, in a real scenario, bacteria may become lost or die during the transmission. In order to account for these effects, we assume that, if the bacterium does not reach the receiver after a certain time t_{max}, it is lost. Then, taking into account that the propagation time can be approximated
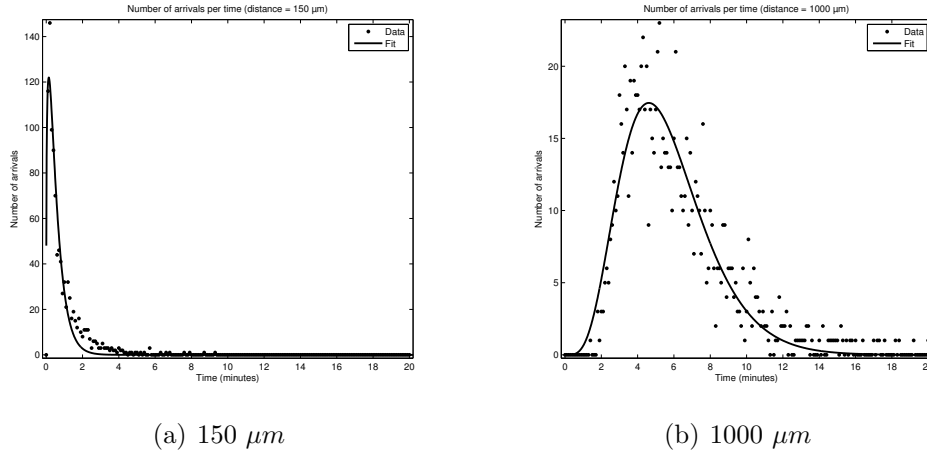
(a) 150 μm (b) 1000 μm

Figure 13: Distribution of arrivals per time for two distances (150 μm and 1000 μm)

by a gamma distribution, the packet loss probability can be approximated as:

$$P_{loss}(d) = P[(t_{prop}(d) > t_{max}] = 1 - F(t_{max}, k, \theta) = \sum_{i=0}^{k-1} \frac{(t_{max}/\theta)^i}{i!} e^{-t_{max}/\theta}$$

(21)

where F is the cumulative distribution function of the gamma distribution. Taking this into consideration, the amount of losses increases as the distance increases.

7. Conclusions

In this paper we have proposed a new mechanism for medium-range communications in nano-networks: flagellated bacteria. This method is based on encoding the information in DNA sequences (a DNA packet) and transmitting it using a flagellated bacterium as a carrier. In the proposed technique the receiver is constantly releasing attractants to the environment that are sensed by the bacteria that in turn, propel themselves towards the receiver.

We have presented a physical channel characterization for flagellated bacteria in nano-networks. To this aim, we have modeled the diffusion of attractants, the biased random-walk of bacterium and its impulse-response to the attractants. Finally we have developed a simulator that allows us to

characterize both the propagation delay and the packet loss probability. Researchers working in novel nano-network architectures may consider these results when designing new protocols or architectures for nano-networks.

References

[1] D.J. Schurig et al., "Metamaterial Electromagnetic Cloak at Microwave Frequencies" Science, vol. 314, no. 5801, pp. 977 - 980, Nov 2006.

[2] I.F. Akyildiz, F. Brunetti, and C. Blázquez, "Nanonetworks: A new communication paradigm" Computer Networks, vol. 52, no. 12, 2008.

[3] H. Goldstein, "The race to the bottom [consumer nanodevice]" Spectrum, IEEE, vol. 42, no. 3, pp. 32-39, March 2005.

[4] M. Meyyappan, J Li, J Li, and A. Cassell, "Nanotechnology: An Overview and Integration with MEMS" in Proceedings of the 19th IEEE International Conference on Micro Electro Mechanical Systems, Istanbul, 2006, pp. 1-3.

[5] K.E. Drexler, "Nanosystems: molecular machinery, manufacturing, and computation" New York: John Wiley & Sons, 1992.

[6] M. Gregori, I. F. Akyildiz, "A New NanoNetwork Architecture using Flagellated Bacteria and Catalytic Nanomotors", to appear in IEEE Journal on Selected Areas of Communications 2010,

[7] F.R. Blattner et al., "The Complete Genome Sequence of Escherichia coli K-12" Science, vol. 277, no. 5331, pp. 1453 - 1462, September 1997.

[8] D.L. Nelson and M.M. Cox, "Lehninger principles of biochemistry" 4th ed.: W. H. Freeman and Company, 2005.

[9] I.S. Johnson, "Human Insulin from Recombinant DNA Technology" Science, New Series, vol. 219, no. 4585, pp. 632-637, Feb 1983.

[10] D.A. Fletcher and J.A. Theriot, "An introduction to cell motility for the physical scientist" Phys Biol., vol. 1, no. T1-T10, June 2004.

[11] G.L. Hazelbauer, R.E. Miesibov, and J. Adler, "Escherichia coli mutants defective in chemotaxis toward specific chemicals" Proceedings of the National Academy of Sciences of the United States of America (PNAS), vol. 64, no. 4, pp. 1300-1307, December 1969.

[12] R.J. Lipton and E.B. Baum, "DNA Based Computers" in Proceedings of a Dimacs Workshop April 4, 1995. Princeton University: AMS Bookstore, 1996.

[13] G. Lipps, "Plasmids: Current Research and Future Trends" Georg Lipps, Ed.: Caister Academic Press, 2008.

[14] J. Adler, "Chemotaxis in Bacteria" Annual Review of Biochemistry, vol. 44, pp. 341-356, 1975.

[15] H.C. Berg and D.A. Brown, "Chemotaxis in Escherichia coli analysed by three-dimensional tracking" Nature, vol. 239, Oct 1972.

[16] K.E. Drexler, "Nanosystems: molecular machinery, manufacturing, and computation" New York: John Wiley & Sons, 1992.

[17] J. Adler, "Chemoreceptors in Bacteria" Science, vol. 166, no. 3913, pp. 1588-1597, Dec 1969.

[18] N. Rott, "Note on the History of the Reynolds Number" Annual Review of Fluid Mechanics, vol. 22, pp. 1-11, 1990.

[19] E.M. Purcell, "Life at Low Reynolds Number" American Journal of Physics, vol. 45, pp. 3-11, 1977.

[20] H.C. Berg, "*E. coli* in Motion: Biological and Medical Physics Biomedical Engineering" Elias Greenbaum, Ed.: Springer, 2004.

[21] J. Philibert, "One and a half century of diffusion: Fick, Einstein, before and beyond" Diffusion Fundamentals, vol. 2, pp. 1.1-1.10, 2005.

[22] M. Pierobon and I.F. Akyildiz, "A Physical Channel Model for Molecular Communication in Nanonetworks" in IEEE Journal on Selected Areas in Communications (JSAC) on BIO-INSPIRED NETWORKING, 2010.

[23] V. Sourjik and H.C. Berg "Receptor sensitivity in bacterial chemotaxis" PNAS, vol. 99, no. 1, pp. 123-127, January 2002.

[24] H.C. Berg, "Random walks in biology" Princeton University Press, 1993.

[25] S.M. Block, J.E. Segall, and H.C. Berg "Impulse Responses in Bacterial Chemotaxis" Cell, vol. 31, pp. 215-226, November 1982.

[26] E. Drexler, "Nanosystems: Molecular Machinery, Manufacturing, and Computation", John Wiley and Sons Inc., 1992.

[27] T. Nakano, T. Suda, M. Moore, R. Egashira, A. Enomoto, and K. Arima, "Molecular communication for nanomachines using intercellular calcium signaling in 5th IEEE Conference on Nanotechnology, vol. 2, pp. 478481, July 2005.

[28] M. Moore, A. Enomoto, T. Nakano, R. Egashira, T. Suda, A. Kayasuga, H. Kojima, H. Sakakibara, K. Oiwa, "A design of a molecular communication system for nanomachines using molecular motors" in Proceedings of the Fourth Annual IEEE International Conference on Pervasive Computing and Communications (PerCom'06), March 2006

[29] Parcerisa L. and Akyildiz, I. F, "Molecular Communication Options for Long Range Nanonetworks" Computer Networks Journal (Elsevier), Vol. 53, No. 16, pp. 2753-2766, November, 2009

[30] Chandrasekhar, S., "Stochastic Problems in Physics and Astronomy" Rev. Mod. Phys., Vol. 15, No. 1, pp. 1-89, January 1943.

[31] Berg, H.C., "The rotary motor of bacterial flagella" Annual Review of Biochemistry, vol. 72, pp. 19-54, 2003.

[32] Vale, R., "The molecular motor toolbox for intracellular transport Cell, vol. 112, pp. 467480, February 2003.

[33] JW Drake, B Charlesworth, D Charlesworth, and JF Crow, "Rates of spontaneous mutation" Genetics, vol. 148, p. 16671686, Apr 1998.

8. Appendix

This appendix details all the parameters used in the simulations and the pseudo-code of the simulator:

- The simulation space is assumed to be a 2D squared space with a length side of 2 mm. This allows us to simulate a range of up to 1mm.

- The transmitter is located in the middle of the space, hence, in the position $x = y = 1$mm.

- The receiver is located in a distance d from the transmitter. Hence, in the position $x = d + 1$mm and $y = 1$mm.

- The bacterium is moving at a constant velocity $v = 20\mu$/s (see [20]).

- Initially the bacterium is in the running state and it is released in the correct direction, that is in the positive direction of the x axis.

- The bacterium reaches the receiver if the distance that separates them is less than $15\ \mu m$.

- The time-step of the simulation is 0.01 s.

State?

Run | Tumble

- Read Concetration
- Calculate Proability of changing to Tumble
- Decide Next State
- Move in direction θ and velocity v.
- Apply Rotational Diffusion (θ= θ+ φ)

- Read Concetration
- Calculate Proability of changing to Run
- Decide Next State

Next State run?

No | Yes

- Calculate change in direction from run to run (θ= θ + γ)

Destination Reached? —Yes— Propagation time = t
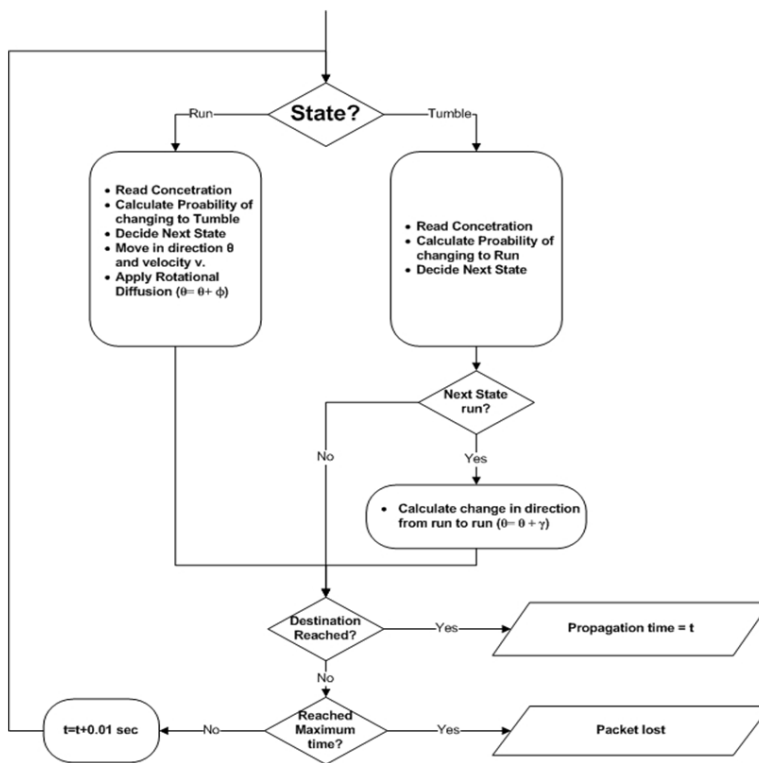
No

Reached Maximum time? —Yes— Packet lost

t=t+0.01 sec —No—

Figure 14: Pseudo-code of the simulator

30