Nano Communication Networks 3 (2012) 36–45

Contents lists available at SciVerse ScienceDirect

Nano Communication Networks

journal homepage: www.elsevier.com/locate/nanocomnet

Opportunistic routing through conjugation in bacteria communication nanonetwork

Pietro Lio' [a], Sasitharan Balasubramaniam [b,*]

[a] The Computer Laboratory, University of Cambridge, UK

[b] Telecommunication Software and Systems Group, Waterford Institute of Technology, Carriganore campus, Waterford, Ireland

ARTICLE INFO

Article history:
Received 7 October 2011
Accepted 8 October 2011
Available online 14 October 2011

Keywords:
Bacteria communication nanonetworks
Opportunistic routing

ABSTRACT

As the field of molecular communication continues to grow, numerous solutions have been proposed to enable communication between nanomachines. Amongst these solutions, bacteria communication nanonetworks has been proposed as a promising approach for molecular communication. This is driven by a number of attractive properties found in bacteria, which includes biased motility toward the destination through chemotaxis process, as well as the ability of bacteria to transfer genetic information between each other using conjugation. Bacterial conjugation is a major mechanism for Lateral Gene Transfer (LGT) that enables information transfer among bacteria. In this paper, we propose an opportunistic routing process in bacteria communication network using these two properties. The paper presents the simulation work to analyze the performance of message delivery for three different topology shapes, which includes grid, hexagon, and T-shape topologies. The aim of simulating on different shape topologies is to determine the impact that conjugation will have to improve message delivery. In all topologies, the use of conjugation helped improve the reliability of message delivery to the destination point. The paper will analyze various commonly used metrics used in communication networks, such as the average delay, the number of messages, as well as the distribution of messages and their originating node. The conjugation process is most beneficial in complexed shaped topologies, where the directionality from the source to the destination is a number of hops apart, as represented in the T-shape topology.

1. Introduction

Nanotechnology has proven to be highly effective in supporting numerous challenging problems, in particular in the healthcare and pharmaceutical industry. The ability to manipulate matter at the micro/nano scale could provide various advantages. From a healthcare perspective, this could include capabilities such as early detection or ability to cure chronic diseases. While there are advantages in nano scale devices, there are also numerous disadvantages. The main disadvantage rises from the sheer size of these devices, which prevents nano scale devices to have sophisticated functionalities and capabilities. However, augmenting these devices with various other functionalities within the environment could help enhance and improve their capabilities. One of these functionalities is communication at the nano/molecular scale [3]. Unlike conventional communication devices, communicating at the molecular scale brings along a number of challenges. First and foremost, the communication between devices must be performed using biological components. The grand challenge in using this approach is the ability to transfer concepts from conventional communication and networking to these biological components. A number of different solutions have been proposed for molecular communication such as calcium signaling [28], neuron

* Corresponding author. Tel.: +353 051 302912.
E-mail address: sasib@tssg.org (S. Balasubramaniam).

1878-7789/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.nancom.2011.10.003

based nanomachine [16], carbon nanotube for ad hoc networks [5], as well as bacteria networks [10]. Besides the use of biological components to create nanonetworks, a number of work have also proposed concepts from communication network for nanonetworks, such as determining the end-to-end capacity for biological diffusion based molecular communication [30] or development of MAC layer protocols [4]. From of these various solutions that are currently being investigated, an interesting approach is using bacteria communication nanonetworks. Bacteria have an interesting inherent characteristic, where motility can be directed through a chemoattractant. At the same time, most bacteria are known to represent easy to grow organisms which can perform thousands of operations per second. An example of a well known and studied bacteria is the *Escherichia coli* (*E. coli*), which are motile rod-shaped cylinders a few microns long. A number of bacterial species have developed different types of complex information passing and communication protocols, also taking into account trust and security issues, which usually involves chemical messengers [26]. An example is given by the quorum sensing process [1] which is important for bacterial colonization and virulence (e.g. biofilm formation, bioluminescence, and secretion of virulence factors).

Recent progress made in the fields of microfluidics [39,15,37], inkjet printing [8,11,25,41], and synthetic biology provide unprecedented experimental methods for manipulating bacterial cell-to-cell communication and sheds light on single cell- and population-level information processing. Molecular biotechnology today enables relatively cheap and simple to develop design automation of bacterial genes that could modify internal signaling networks, inter-bacterial communication signaling, as well as the capability of transferring DNA-messages and guide their integration in the host [12,27]. Such artificial manipulations of bacterial signaling could be beneficial in development and design of future Microbial biofilm or microfluidic devices. Microbial biofilms are formed by diverse yet highly-structured layered communities living within the confines of sharply-defined laminated biochemical gradients that predictably fluctuate during the day [9,24,2,33]. Here we focus on bacterial properties which may be more suitable for microfluidic devices as they take advantages of the variety and the mechanisms of bacterial motility.

Bacteria swim by means of thin helical flagella on their surface driven by molecular motor machines embedded in the bacterial membrane; some species as for example *bdellovibrio* could swim at the speed of 160 μm/s. The flagella rotate at speeds of more that one hundred cycles per second, where the motors can sporadically stop and start, change the bundle arrangements of the flagella, and therefore, their direction of rotation which steers the bacteria according to the surrounding environment. Bacterial motility is essential for mobilizing the bacterium across a chemoattractant gradient or away from a poisonous chemical. For example *E. coli* can detect more than 50 distinct chemicals producing chemoattractants, and repellents even at concentration of less than one part in ten million. These sensorial abilities and others, such as capabilities to survive from antibiotics, poisonous environment, etc., have evolved both through the modification of existing sequences (DNA based mutations) or through Lateral Gene Transfer (LGT), which is a process of genetic material exchange (this process is also known as bacteria conjugation). The availability of complete genomic sequences has recently provided an opportunity to measure the cumulative amount of laterally transferred sequences in diverse bacterial genomes [31,29] and allow to infer the potentialities of improving a bacterium by leveraging on billion years evolution occurred in other microorganisms.

In this paper, we focus on discussing how the bacterial conjugation process could be efficiently exploited for routing in bacteria communication nanonetworks. Conjugation is the process of transferring DNA from a donor to a recipient cell with the establishment of the incoming DNA and its cargo of genetic traits within the transconjugant [36,13]. The advantage of conjugation is the integration of foreign genetic into the recipient bacterium, which can acquire new abilities. While the advantage seems attractive, the disadvantage of conjugation is the slow process due to the requirements of complex protein machinery and sometimes security and trust procedure that reside in the recipient bacteria [31,13]. While most microfluidic or printing solutions to date have concentrated on single hop transmission of bacteria communication, the distance is a limiting constraint. In order to support long distance communication, a multi-hop approach maybe required. However, trying to implement a multi-hop routing mechanism in bacteria nanonetwork is a challenge, since bacteria networks are not as flexible as conventional routing system, where software could be changed and modified in nodes to implement specific routing functionality. In order to develop routing capability for bacteria communication nanonetworks, we apply the bacterial conjugation process. Applying both bacteria chemoattractants and conjugation enable us to implement the Delay Tolerant Network (DTN) routing mechanism in bacteria nanonetworks. DTN [17] provides capabilities for wireless devices, in particular mobile devices, to opportunistically communicate between nodes depending on the current environment (e.g. signal strength, location). In [19], Hui et al. investigated DTN that is based on social ranking between members of a social group. As a mobile device encounters another device within its vicinity, it checks the social ranking of the owner, and if the ranking is higher the message is passed on to that device. The aim is to allow users with high sociability to be the carrier of the message, in order to increase the probability that a message will reach its destination. In this paper, we have taken a similar approach to transfer messages between bacteria to support long distance transmission. In our scenario, each bacteria mimics a user carrying a mobile device, and transfers message between bacteria through the conjugation process. Bacteria will migrate toward chemoattractants (their source co-locate with the access point), and as they encounter bacteria migrating toward a different chemoattractant, a message is transferred. We have performed extensive simulation to validate our proposed approach, in particular for three different topology shapes. Performance evaluation have also been performed, to determine the success in message delivery, the average delay, as well as impact of conjugation in enabling reliability of message transfer.

This paper is organized as follows: Section 2 provides background information on bacteria and the conjugation

process, while Section 3 describes our proposed approach for opportunistic routing in bacteria nanonetwork. Section 4 presents our simulation work, while Section 5 presents possible applications of bacteria communication nanonetworks for design of future microfluidic devices. Lastly, Section 6 draws conclusions and potentialities.

2. Bacteria communication

2.1. Bacteria communication vs. motility

Cobo and Akyildiz [10] first investigated the use of bacteria for molecular communication between different nanomachines, in particular for point-to-point communication. Bacteria are able to control the random walk of their motion in order to accumulate toward favorable environments and disperse from less favorable environments. The motility of many bacteria such as *E. coli* depends on several helical flagellar filaments, each embedded in the membrane and driven at its base by a reversible rotary motor. We can distinguish two states: when the motors turn counterclockwise (CCW), the filaments form a bundle that propels the bacterium forward. The bundle falls apart when one or more motors turns in the clockwise (CW) direction, and the bacterium tumbles. A new swimming direction is selected upon resuming the CCW rotation of the flagellar motors. The bacterium modulates the CCW and CW intervals according to sensing the external chemical environment. Tumbles are mostly suppressed when cells move up spatial gradients of chemical attractants. The Keller–Segel model uses a set of coupled partial differential equations to describe the mathematics of chemoattraction for population of bacteria by relating bacterial density fluctuations to chemoattractant concentrations [21]. A noteworthy point to be made is that recent chemotaxis models fit very well to the distributions of lengths of "runs" (CCW) and "tumbles" (CW) observed in experimental data [7]. The *E. coli* chemotaxis behavior is supposed to be formed by two coupled modules, which are the receptor and adaptation module. The receptor module detects changes in the environment which are transduced internally. The adaptation module acts in maintaining the intracellular signal at a steady state that is indifferent to ambient concentrations of the chemoattractant [34]. Dependences of the motor switching rates from the motor torque have been investigated for near zero to high loads [42]. There are also important technological breakthroughs in the direction of biotechnology exploitation of chemotaxis. For example, Kovarik et al. have described a microfluidic device that establishes a stable chemical gradient for chemotaxis assays in less than 1 min [23]. Kim et al. have built a long-range concentration gradient generator device that produces multiple linear concentration gradients simultaneously on a single chip [22]. They found that the motion of bacteria generates bands migrating toward higher concentrations of the chemoattractant (a sugar) until the consumption rate by the bacteria and the diffusion rate of the chemoattractant are in equilibrium. They also determined that *E. coli* prefers glucose, galactose, and mannose to arabinose and xylose, in this order.

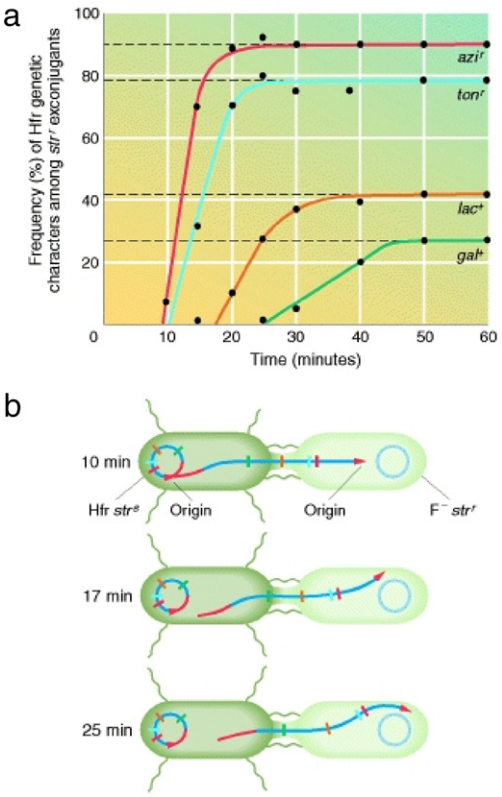

Fig. 1. (a) Conjugation with respect to time [40], (b) an illustration of conjugation between two bacteria.

2.2. How bacteria transfer information

As described in the introduction, conjugation enables a process for bacteria to pass the genetic information from one bacteria to the next (Fig. 1(b)). Bacterial conjugation involves the transfer of single-strand DNA (ssDNA, or in some cases, double strand DNA, dsDNA) between donor and recipient cells that are normally in close contact. The effectiveness of conjugation in disseminating traits such as antibiotic resistance is remarkable, with resistance to newly available antibiotics appearing within months of their introduction [14]. Conjugation is mediated by self-transmissible plasmids such as F-Plasmids, as well as phage-like sequences that have been integrated into the bacterial chromosome, such as Integrative and Conjugative Elements (ICEs). Both conjugative plasmids and ICEs can mediate the transfer of mobilizable elements by sharing their conjugative machinery. An important observation to be made is that many bacteria grow in chains, i.e. dense communities of cells, where the presence of conjugative elements in cells can contribute to the formation of such communities; Babic et al. found that when acquired by one cell in a chain, ICEs spread rapidly from cell to cell within the chain by additional sequential conjugation events [6]. This intra-chain conjugation is inherently more efficient than conjugation that is due to chance encounters between individual cells. Therefore, although the process is slow because it requires building a protein complex, it can quickly spread, where a single donor cell

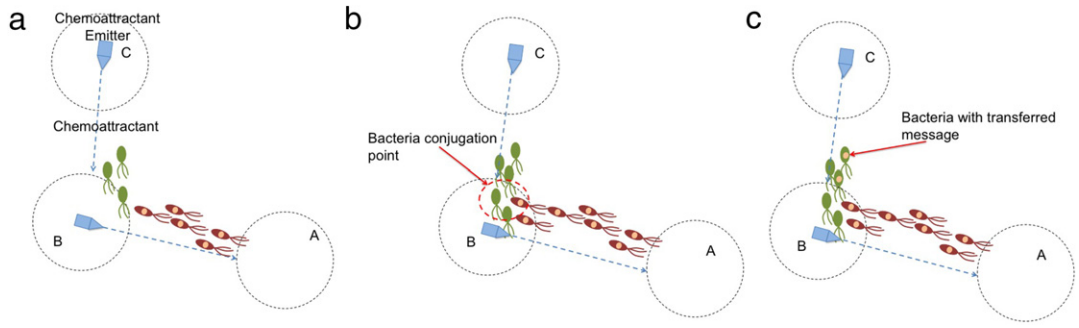

Fig. 2. Opportunistic routing using bacteria conjugation, (a) bacteria are slowly released from each node, (b) the different bacteria are encountered, and (c) the conjugation process is complete, where the information message has been transferred.

can convert a population of recipient cells to donor cell status via a process similar to epidemic spreading. Conjugation requires coupling proteins that links the transferosome (a type IV secretion system in Gram-negative bacteria) to the relaxosome, a nucleoprotein complex at the origin of transfer (*oriT*). Although broadly speaking, the transfer systems appear to be able to drill a hole through any recipient cell envelope and start the DNA transfer in a recipient-independent manner, there are several factors and security check that affect the process. The transfer potential of these transfer regions depends on the integration of many signals in response to environmental and physiological cues. Conjugative elements can be narrow or broad host range, depending on their ability to be established and maintained in the new host. Genetic information transfer could be modulated by repressors and activators, which can induced via small molecules or peptides, or in response to excision from the host genome. Plasmids have different optimal range of temperature. F-like plasmids have an optimum temperature for mating of approximately 37 °C, and mating is almost undetectable at high (50 °C) temperatures. An intriguing mechanism for blocking DNA transfer between two related donor cells is entry exclusion (*Eex*) with many conjugative systems encoding Eex genes as well as associated genes for surface exclusion (*Sfx*), which block cell-to-cell contact [31,13,32]. It is noteworthy that bacteria such as *Enterococcus* use pheromones to trigger gene expression prior to conjugative DNA transfer with the pheromone being released by the recipient cell. Therefore both temperature and pheromones could be used in a device to modify the conjugation rate.

Conjugation depends on the amount of information on the gene, where the longer the gene, the longer the conjugation process. The quantity of genetic information transferred during the conjugation process, with respect to time is presented in 1(a). The relationship between time and amount of transferred genetic information is linear (after a certain delay due to the assembling of the protein machinery) and accurate enough to be used for identifying the order of the genes through so called interrupted-mating (Jacob and Wollman [20]). The transfer process takes several minutes to start due to the assembling of the related structures, but proceeds quickly. For example, a sequence of one hundred bases could take 5 min for the transfer, while 5 million bases will take approximately 100 min. The other important property of conjugation is the meeting distance between the bacteria. The surface of the bacteria contains pili, which allows the bacteria to come together and form contacts. The P and type pili are 1 μm long and 6–7 nm diameter; they have extension speed of 400 nm/s and 6 nm/s. However, the pili is not used as a tube to transfer the plasmid, but rather a point of contact, where the contact point will draw the two bacteria together, after which transfer of replicated plasmid is performed. In many cases bacteria form colonies and biofilms which make the encounters highly probable.

3. Multi-hop nodes and opportunistic routing in bacteria nanonetworks

The description of the conjugation based opportunistic routing for bacteria nanonetworks is illustrated in Fig. 2, and the details of the process will be described in this section.

3.1. Node configuration

The nanomachine (from hereon, this will be referred to as a node) configuration for our proposed solution is illustrated in Fig. 2. We assume that each nanomachine is able to house a chemoattractant emitter that emits chemoattractant to attract bacteria from the neighbor nodes. Each node will also house bacteria that will be attracted to the chemoattractant of the neighbor node. Therefore, an important assumption is that the bacteria chemoreceptors of a node must match the chemoattractant of the neighbor node.

3.2. Conjugation based routing

We assume that the bacteria used as the carrier of information is the *E. coli* bacteria. Fig. 2 illustrates the process of multi-hop routing in bacteria communication. As shown in the figure, the process involves two bacterial properties, which are chemotaxis and conjugation. We make a number of assumptions in our proposed solution, which are as follows: (i) each node contains a number of sub-nodes, where each of these nodes can emit both chemoattractant as well as bacteria, (ii) we assume that there is a process for uploading information onto the bacteria, and (iii) we assume that each of these nodes are fixed and anchored.

Table 1
Simulation parameters.

Parameter	Value
Bacteria motility	
Speed	20 μs
Run duration toward the destination	Exponential distribution ($\lambda = 1/7.5$)
Run duration away from destination	Exponential distribution ($\lambda = 1/3.5$)
Tumbling angle	Normal distribution (0–360)
Direction of tumbling	Coin flip
Number of chemoattractants	1
Conjugation process	
Conjugation time	300 s
Message length	20 base pair
Environment	Constant temperature (37 °C)
Receiver	
Receiver radius	2 μm
Attractant emission rate (Q)	10^{-12} mol/s
Diffusion coefficient (D)	10^{-9} m^2/s

Initially we assume that each nodes emit a chemoattractant compound that only spreads as far as the neighbor node. At the same time, the paths of bacterial motility should cross or be close to each other. For example, the green bacteria in Fig. 2 are attracted to the chemoattractant emitted by node C, while the red bacteria are attracted to the chemoattractant emitted by node B (the different colors of the bacteria represents the different chemoreceptor types on the bacteria). Therefore, as we can see the two different types of bacteria will cross at a specific junction. During this crossing period, bacterial conjugation occurs. At these conjugation points, the messages of bacteria from node A can be transferred to the bacteria traveling from B to C. Therefore, we are able to gain opportunistic like routing for 2 hop bacteria nanonetwork, where messages originating from node A will eventually arrive at node C.

The drawback of this, is of course, the fact that messages will be transmitted to numerous bacteria. In part, this is to ensure a certain guarantee of messages will arrive at the destination, since encounters are probabilistic and the conjugation process could be low. However, our proposed solution could be applied to numerous other types of bacteria that have different encountering properties. For example, *Bacillus subtilis* (*B. subtilis*), may grow in chains often in communities of cells. Therefore, a single initial successful event will allow information transfer to many bacteria within the chain, where the recipient maybe contained within the chain.

4. Simulation

In this section we will discuss the simulation that was performed to validate the opportunistic routing process through bacterial conjugation. We will first present the simulation setup and this will be followed with the discussion on the results obtained.

4.1. Simulation setup

Table 1 presents the parameters that were used for our simulation. The behavior of bacteria motility is an interchange between two states, which is the running state or the tumbling state. Our simulation of bacteria motility and node arrangement is based on 2-dimensional medium. We based our bacteria motility on the similar approach that was used by [38], where the bacteria exhibit a random walk behavior dependent on their orientation and direction of motility toward the destination. This is reflected in the parameters presented in Table 1, where the random walk mobility is based on an exponential distribution, and has a different λ value between moving toward or away from the destination. The decision to change its angle between clockwise and anti-clockwise, is based on a binary coin flip, and the angle of tumbling is based on a normal distribution. As described earlier, the conjugation process depends largely on the distances between the bacteria. There are a number of assumptions made in our simulation for the conjugation process. Although conjugation process is a lengthy process, the majority of the time is spent for the different bacteria to form the pili before the conjugation process takes place. We assume in our case, that the pili are already formed on the bacteria, and so during the encountering process, the bacteria could instantly start the conjugation process. This assumption corresponds to keep high intracellular levels of the proteins involved in the process. We assume that the conjugation process will occur once the two bacteria cells approach each other on the same location, which represents a constant temperature that supports maximum conjugation rate. Once two bacteria approach the same point, a conjugation process occurs for 300 s (in section B, we will simulate for varying conjugation time), which amounts to transfer of 20 base gene length. Here, to shrink times a little, besides the pre-formed pili, we also assume that most of the protein complex machinery are in a pre-assembled conditions or are ready, i.e. the bacteria have been alarmed with a pheromone; from a biotechnology point of view, our assumption corresponds to an increase expression and deregulation of the genes coding for the machinery. We use this static conjugation time value for all the simulation scenarios. During the conjugation process, the bacteria will remain in the same location, and will only be mobile again once this process is complete. We assume a constant stationary level of chemoattractant, which is represented as $U(r, t) = \frac{1}{1000}\frac{Q}{4D\pi r}$. The parameters for chemoattractant

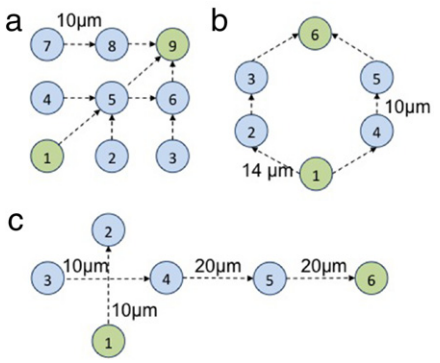

Fig. 3. Topologies used for simulation (a) grid (Topology 1), (b) hexagon (Topology 2), (c) T-shape (Topology 3).

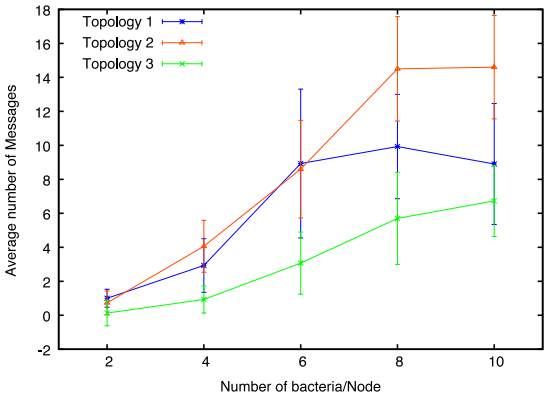

Fig. 4. Number of successful messages vs. number of bacteria emitted per node.

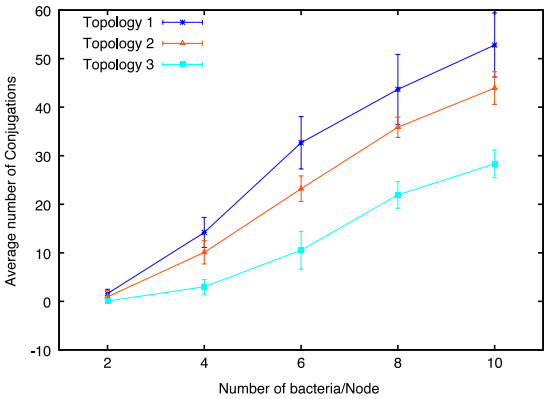

Fig. 5. Number of conjugation performed vs. number of bacteria emitted per node.

concentration can be found in Table 1. As stated in [10], the Q value of 10^{-12} mol/s usually leads to saturation if the bacteria is less than 4 cm away from the destination. However, this does not affect the biased motility that the bacteria will have as it mobilizes toward the destination node.

The topologies that we used in our simulations are illustrated in Fig. 3, which includes a grid (Topology 1), hexagon (Topology 2), as well as a T-shape (Topology 3).

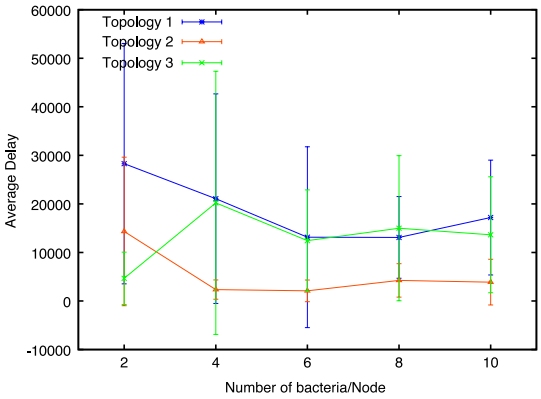

Fig. 6. Average delay before 1st message arrived at destination.

The green nodes indicate the source and destination nodes (for all topologies, the source is node 1). Our intention is to determine the impact that each topology shape will have on the conjugative process, and how this in turn will affect the message delivery performance. In the case of the grid topology, the directionality of the chemotaxis gradient is biased toward the destination node. The aim for this specific topology is to determine, how bias migration of the bacteria toward a specific direction could accumulate the bacteria to perform conjugation in a certain location, and how this enhances the reliability. The aim of the hexagon shape topology is to determine how the two possible routes toward the destination could impact on the choice of motility, and in turn how they would influence the number of conjugations. The T-shape topology was created to have the chemoattractant of the source node to flow in a different direction to the destination node. The objective is to allow maximum conjugation to occur between bacteria flowing from node 1 to 2, and from node 3 to 4 (the chemoattractant will cross with each other). The conjugation at this crossing point, will determine how many bacteria carrying the message will eventually move toward the direction of the destination node. While *E. coli* are capable of sensing up to 50 different attractants, in our simulation we assume that each bacteria is equipped with a single chemoreceptor [10], and is attracted to a single chemoattractant that is emitted by the immediate neighboring node. Therefore, as described in the earlier section, we assume that each bacteria will only be attracted to its one hop neighbor. The arrows between the nodes in Fig. 3, illustrates the direction of chemoattractant from its neighboring node.

4.2. Fixed conjugation period

In this first section of the simulation, we assume a fixed period of conjugation between the bacteria. The results of our simulation is presented in Figs. 4–8. Fig. 4 presents the number of messages that have successfully arrived at the destination, while Fig. 5 presents the number of conjugation that is performed with respect to the number of bacteria emitted per node. We evaluated the performance of the message delivery with respect to the number of bacteria emitted per node (2–10). As predicted,

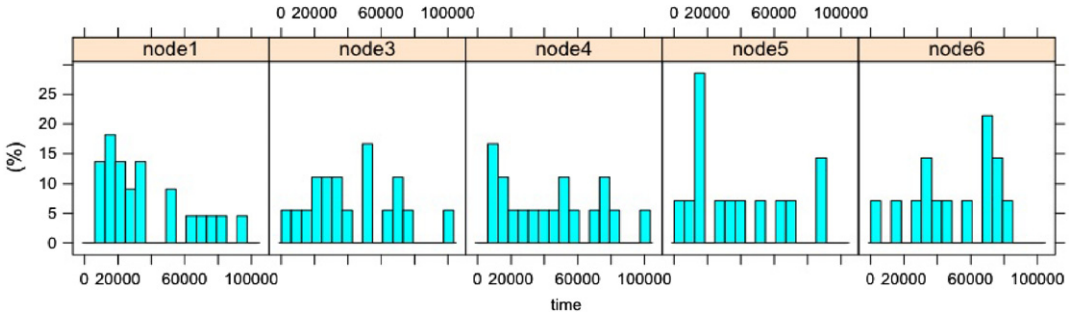

Fig. 7. Arrival distribution of bacteria with respect to originating node for Topology 3, (a) node 1, (b) node 3, (c) node 4, (d) node 5, (e) node 6.

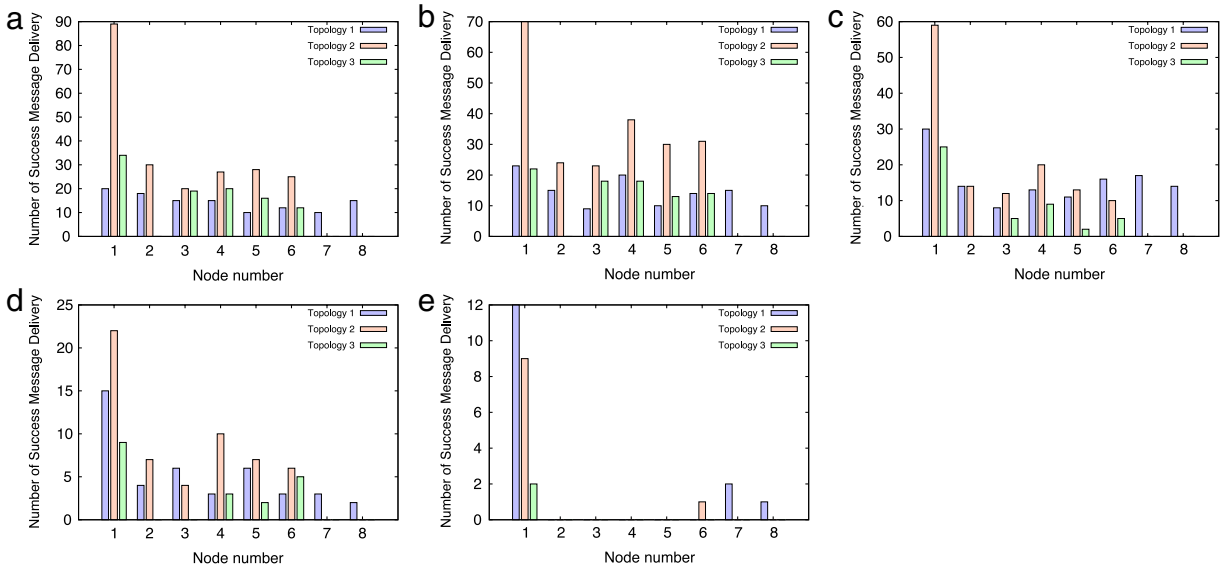

Fig. 8. Number of bacteria arriving at the destination with respect to node number, (a) 10 bacteria/node, (b) 8 bacteria/node, (c) 6 bacteria/node, (d) 4 bacteria/node, (e) 2 bacteria/node.

the number of successful messages arriving at the destination increases as the number of bacteria per node increases. This is largely due to the increase probability of conjugation, as well as numbers of bacteria that will reach the destination. As far as the number of messages successfully delivered to the destination is concerned, we can see that Topology 2 outperforms topologies 1 and 3, in particular, when the number of bacteria per node is high (average message delivery is 14.6 for Topology 2 compared to 6.73 for Topology 3 and 8.9 for Topology 1—for 10 bacteria emitted/node). This performance is largely attributed to the shape of Topology 2. As shown in Fig. 3(b), the bacteria emitted from node 1 are attracted to the chemoattractants of nodes 2 and 4. While most bacteria are biased toward this direction, we have found that a number of bacteria have migrated through the hollow center of the topology to reach its destination at node 6. This, has also been shown in number of conjugations that have been performed (Fig. 5), which is lower than Topology 1, since most of the bacteria have migrated directly to node 6 and bypassing the other intermediary nodes (average number of conjugation is 43.93 for Topology 2 compared to 28.33 for Topology 3). The histogram in Fig. 8, which shows from which node the bacteria that successfully delivered the message originated from, reiterates this, where we can see that majority of the successful messages arrived from node 1 for all number of bacteria per node. For the entire 15 run of the simulation, up to 89 messages arrived from bacteria that originated from node 1 in Topology 2 for 10 bacteria/node. However, Topology 1 and 3 in Fig. 8(a) shows a more even spread of the messages that arrived from bacterias that originated from other nodes. The average delay in Fig. 6, also shows that Topology 2 had the lowest delay for the first message to arrive at the destination, where for 10 bacteria/node the average for Topology 2 was 3882.9 s, while for Topology 1 is 17,188.25 s and Topology 3 is 13, 642.73 s. This large gap in the delay could be attributed to the conjugation process, since the bacteria will be stationary during that process.

On the other hand, the grid configuration of Topology 1, induces a higher number of conjugation process, as the bacteria are biased toward the destination of node 9. Since most bacterias are biased toward this direction, we start to see a large density of bacteria moving toward the destination, which in turn induces higher number of conjugation. According to Fig. 8, the number of messages arriving from the different nodes, is evenly spread

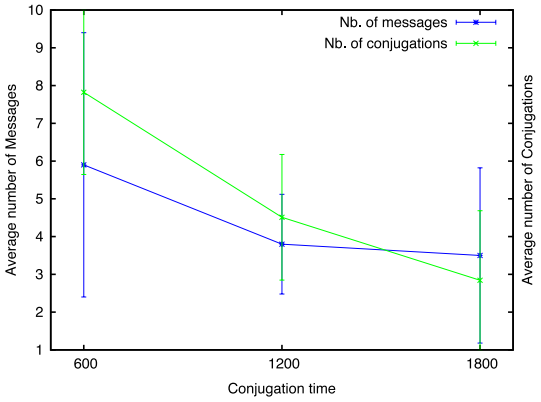

Fig. 9. Arrival number of successful messages with respect to varying conjugation time.

(in particular for higher number of bacteria per node as shown in Fig. 8(a), where we see on average about 12–20 number of successful messages arrive at the destination). As we move toward lower number of bacteria per node, we start to see that most of the messages are arriving from the source node. However, the benefits of the conjugation process is also demonstrated in the average delay of Fig. 5, which shows that as the number of bacteria per node increases, the average delay starts to decrease. The Topology 3 is designed to test the benefits of conjugation, in particular, if the destination node is in a different direction from the bacteria that are emitted from the source node. As shown in Fig. 3(c), the bacteria carrying the message emitted from node 1 is attracted to node 2, which is 90 degrees anti-clockwise from the direction of destination node 6. We have also deliberately placed node 5 and 6 slightly further away from node 4. While Fig. 4 shows that Topology 3 has the lowest average for number of successfully delivered messages, Fig. 8 shows that most of the messages arriving to the destination are evenly spread between all the nodes (once again, particularly for higher number of bacteria per node). Fig. 7 shows the distribution of messages arriving from each node with respect to time. We can see that a good percentage of messages arrived from node 5 (27% of messages arrive between 0–20,000 s) in a very short period of time to the destination, which validates our prediction that the conjugation process is beneficial for this type of topology. This claim is also supported from the results of node 4, which shows that up to 15% of messages from this node arrived at the destination below the time of 10,000 s. Therefore, the performance of the three topologies has shown that, the conjugation contribution is most suited to Topology 3 and 1, and contributes the least to Topology 2. In particular, for Topology 3, the benefits of the conjugation process has been shown through the timely arrival of messages before bacteria from node 1. The benefits of the conjugation process for Topology 1 and 3 has also increase the reliability of information transfer to the destination.

4.3. Varying conjugation period

In this section, we will present the results for varying conjugation period, and the simulation was only performed for Topology 3. As described earlier, the quantity of messages that can be transferred between the bacteria is highly dependent on the conjugation time. In this section we increased the conjugation time to see how this impacts on the success of message delivery to the destination. Fig. 9 presents both the average number of messages successfully delivered to the destination, and the average number of conjugation. Intuitively, we can see that the number of messages that arrives to the destination decreases as the conjugation time increases, and this observation parallels with the number of observed conjugations. Fig. 10 shows the number of successful messages that arrive at the destination with respect to the originating node of the bacteria that delivered the message. We can see that as the conjugation time increases, more messages are arriving from the source node 1. The results have shown that, longer conjugation time will have an impact on the timely delivery of the messages. This is mainly due to the fact that during the conjugation period, the bacteria are stationary until the conjugation process completes. Therefore, during long conjugation time, the bacteria from node 1 will eventually reach the destination before messages from bacteria that went through conjugation.

The simulation work that we have presented in this paper, is only a subset of results to validate the concept of conjugation for routing in bacteria communication nanonetworks. The simulation could be further enhanced to show various parameters and dimension changes (e.g. changes in distances between the nodes), as well as the types of bacteria used for information carrier.

5. Bacterial conjugation in microfluidic devices

We believe there are similarities between the growing interest in building microdevices based synthetic biology

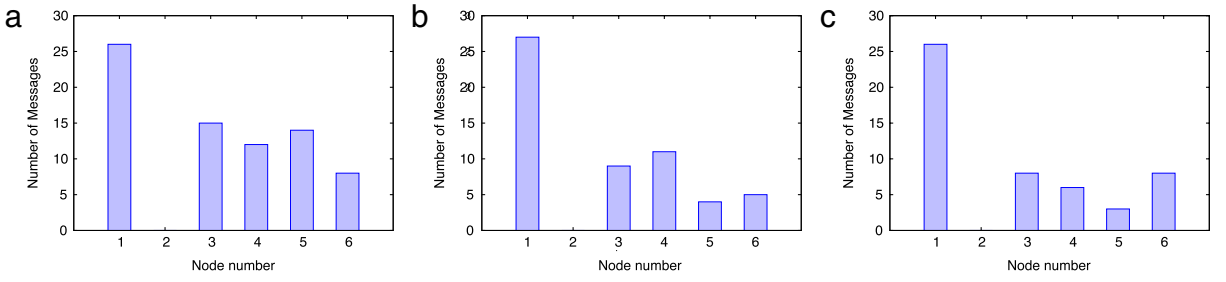

Fig. 10. Number of messages arriving at the destination with respect to originating node for varying conjugation time, (a) 600 s conjugation time (b) 1200 conjugation time (c) 1800 conjugation time.

today, and the electronic design automation approaches that started in the fifties. The electronic design automation, synthetic and systems biology, as well as ICT can develop synergies that benefit each other in unexpected ways. We believe that the concept and solution that has been presented in this paper, will help improve capabilities of nanonetworks, as we start to transfer knowledge from conventional communication systems to molecular nanonetworks. This transfer of knowledge could benefit various different applications, where one of these applications are in microfluidic devices. In a number of microfluidic devices, microactuation is a crucial element in delivering the high capabilities and benefits of these devices. While a number of research work in the past have investigated the use of external devices for microactuation, this defeats the purpose of designing devices at maximum miniaturization. Therefore, a number of works have turned toward alternative solutions to further miniaturize these devices, and one approach that has been proposed is through biological organisms. A good example is the work proposed by Steager et al. [35], who proposed the use of flagellated bacteria as microactuators, where the flagella were able to propel the microstructure through the control of UV light. Another good example is a microrotary motor composed of a 20 μm-diameter silicon dioxide rotor that is driven on a silicon track by the gliding bacterium *Mycoplasma mobile* [18]. Therefore, enabling multi-hop routing within these microfluidic devices could open up numerous opportunities for more sophisticated microfluidic devices. This may include allowing more complex design of rotors and machinery that could be controlled through an overlay network of bacteria communication nanonetworks, where the bacteria maybe able to migrate and dynamically move in response to changes in chemoattractant directions.

6. Conclusion

In this paper, we have proposed an opportunistic routing process for multi-hop bacteria communication nanonetworks. The solution is based on two inherent properties of bacteria, which includes the process of chemotaxis as well as conjugation. Chemotaxis allows the bacteria to have biased random motility to its destination point, while the conjugation process enables bacteria to transfer genetic information from one to another. The conjugation process is highly dependent on a number of factors, such as the pheromone level as well as the environment temperature. We have performed a series of simulation to validate our solution for three different types of topologies (grid, hexagon, and T-shape). The aim of the simulation is to determine the impact that conjugation process will have on enabling successful message delivery, and what this impact will have on the different topology shapes. Our simulation has shown that the conjugation process is most beneficial for nodes that are a distant away, where the originating node has a chemoattractant direction that is in a different direction from the destination (this is in particular for the T-shape topology (Topology 3)). The simulation test has also shown that shorter conjugation process helps to improve the timely delivery of messages, as well as improve the reliability for message delivery. While numerous solutions have proposed the use of pheromones to direct routing, in this paper we propose the use of conjugation as a process for opportunistic routing. The solution that has been proposed in this paper, shows how concepts from communication networking can be transferred to bacteria communication nanonetworks, where this solution could benefit the design of future microfluidic devices. We also believe that this new direction, would help foster new research in routing mechanisms for molecular communications.

Acknowledgment

Sasitharan Balasubramaniam has received support from the Science Foundation Ireland under Grant Number 09/SIRG/I1643 (A Biologically inspired framework supporting network management for the Future Internet). Pietro Lio' was supported by the project RECOGNITION: Relevance and Cognition for Self-Awareness in a Content-Centric Internet (257756), which is funded by the European Commission within the 7th Framework Programme (FP7).

References

[1] S. Abadal, I.F. Akyildiz, Automata modeling of Quorum Sensing for nanocommunication networks, Nano Communication Networks 2 (1) (2011) 74–83.
[2] T. Ahmed, T.S. Shimizu, R. Stocker, Microfluidics for bacterial chemotaxis, Integrative Biology 2 (11–12) (2010) 604–629.
[3] I.F. Akylidiz, F. Brunetti, C. Blazquez, NanoNetworking: A Communication Paradigm, Computer Networks 52 (120) (2008).
[4] B. Atakan, O.B. Akan, Single and Multiple-Access Channel Capacity in Molecular Nanonetworks, in: Proc. ICST/ACM Nano-Net 2009, Luzern, Switzerland, October 2009.
[5] B. Atakan, O.B. Akan, Carbon nanotube-based nanoscale ad hoc networks, IEEE Communications Magazine 48 (6) (2010) 129–135.
[6] A. Babic, M.B. Berkmen, C.A. Lee, A.D. Grossman, Efficient gene transfer in bacterial cell chains, mBio 2 (2) (2011).
[7] D. Bray, M.D. Levin, K. Lipkow, The chemotactic behavior of computer-based surrogate bacteria, Current Biology 17 (2010) 12–19.
[8] P. Calvert, Printing cells, Science 318 (2007) 208–209.
[9] K.C. Cheng, A. Demirci, J.M. Catchmark, Advances in biofilm reactors for production of value-added products, Applied Microbiology and Biotechnology 87 (2) (2010) 445–456.
[10] L.C. Cobo, I.F. Akyildiz, Bacteria-based communication in nanonetworks, Nano Communication Networks, in press (doi:10.1016/j.nancom.2010.12.002).
[11] D.J. Cohen, R.C. Morfino, M.M. Maharbiz, A modified consumer inkjet for spatiotemporal control of gene expression, PLoS One 4 (9) (2009).
[12] Natalie Eynard, Justin Teissie Electrotransformation of Bacteria, 1st ed., Springer, 2010.
[13] L.S. Frost, G. Koraimann, Regulation of bacterial conjugation: balancing opportunity with adversity, Future Microbiology 5 (7) (2010) 1057–1071.
[14] R. Gehring, P. Schummb, M. Youssef, C. Scoglio, A network-based approach for resistance transmission in bacterial populations, Journal of Theoretical Biology 262 (2010) 97–106.
[15] S. Gulati, V. Rouilly, X. Niu, J. Chappell, R.I. Kitney, J.B. Edel, P.S. Freemont, A.J. deMello, Opportunities for microfluidic technologies in synthetic biology, Journal of the Royal Society Interface 6 (2009) 493–506.
[16] A. Guney, B. Atakan, O.B. Akan, Mobile Ad Hoc Nanonetworks with Collision-based Molecular Communication, in: IEEE Transactions on Mobile Computing, (2011) (in press).
[17] K.A. Harras, K.C. Almeroth, E.M. Belding-Royer, Delay tolerant mobile networks DTMNs: controlled flooding in sparse mobile networks, in: Proceedings of Networking, 2005, pp. 1180–1192.
[18] Y. Hiratsuka, M. Miyata, T. Tada, T.Q.P. Uyeda, A Microrotary motor powered by bacteria, Proceedings of the National Academy of Sciences 103 (37) (2006).

[19] P. Hui, J. Crowcroft, E. Yoneki, BUBBLE rap: social-based forwarding in delay tolerant networks, IEEE Transactions on Mobile Computing 6 (1) (2007).
[20] F. Jacob, E.L. Wollman, Genetic and physical determinations of chromosomal segments in Escherichia coli, Symposia of the Society for Experimental Biology 12 (1958) 75–92.
[21] E.F. Keller, L.A. Segel, Model for chemotaxis, Journal of Theoretical Biology 30 (1971) 225–234.
[22] M. Kim, T. Kim, Diffusion-based and long-range concentration gradients of multiple chemicals for bacterial chemotaxis assays, Analytical Chemistry 82 (2010) 9401–9409.
[23] M.L. Kovarik, P.J.B. Brown, D.T. Kysela, C. Berne, A.C. Kinsella, Y.V. Brun, S.C. Jacobson, Microchannel-nanopore device for bacterial chemotaxis assays, Analytical Chemistry 82 (2010) 9357–9364.
[24] P. Landini, D. Antoniani, J.G. Burgess, R. Nijland, Molecular mechanisms of compounds affecting bacterial biofilm formation and dispersal, Applied Microbiology and Biotechnology 86 (3) (2010) 813–823.
[25] J. Merrin, S. Leibler, J.S. Chuang, Printing multistrain bacterial patterns with a piezoelectric inkjet printer, PLoS One 2 (7) (2007) e663.
[26] R.J. Mitchell, S.k. Lee, T. Kim, T.C.M. Ghim, Microbial linguistics: perspectives and applications of microbial cell-to-cell communication, BMB Reports 44 (1) (2011) 1–10.
[27] N.S. Mosier, M.R. Ladisch, Modern Biotechnology: Connecting Innovations in Microbiology and Biochemistry to Engineering Fundamentals, 1 edition, Wiley-AIChE, 2009, 17.
[28] T. Nakano, J.W. Shuai, T. Koujin, T. Suda, Y. Hiraoka, T. Haraguchi, Biological excitable media with non-excitable cells and calcium signaling, Nano Communication Networks 1 (1) (2010) 43–49.
[29] H. Ochman, E. Lerat, V. Daubin, Examining bacterial species under the specter of gene transfer and exchange, in: Proceedings of National Academy of Science. USA, May 3;102 Suppl 1, pp. 6595–6599.
[30] M. Pierobon, I.F. Akyildiz, A physical end-to-end model for molecular communication in nanonetworks, IEEE Journal on Selected Areas in Communications 28 (4) (2010).
[31] O. Popa, T. Dagan, Trends and barriers to lateral gene transfer in prokaryotes, Current Opinion in Microbiology (2011).
[32] D. Prez -Mendoza, F. de la Cruz, Escherichia coli genes affecting recipient ability in plasmid conjugation: are there any? BMC Genomics 10 (2009) 71.
[33] F. Qian, D.E. Morse, Miniaturizing microbial fuel cells, Trends in Biotechnology 29 (2) (2011) 62–69.
[34] T.S. Shimizu, Y. Tu, H.C. Berg, A modular gradient-sensing network for chemotaxis in Escherichia coli revealed by responses to time-varying stimuli, Molecular Systems Biology, 6, 382.
[35] E. Steager, C.-B. Kim, J. Patel, S. Bith, C. Naik, L. Reber, M.J. Kim, Control of microfabricated structures powered by flagellated bacteria using phototaxis, Applied Physics Letters 90 (2007).
[36] E.L. Tatum, J. Lederberg, Gene recombination in the bacterium Escherichia coli, Journal of Bacteriology 53 (6) (1947) 673–684.
[37] G. Velve-Casquillas, M. Le Berre, M. Piel, P.T. Tran, Microfluidic tools for cell biological research, Nano Today 5 (2010) 28–47.
[38] Z. Wang, M. Kim, G. Rosen, Validating models of bacterial chemotaxis by simulating the random motility coefficient, in: Proceedings of 8th IEEE International Conference on BioInformatics and BioEngineering, Athens, Greece, October 2008.
[39] D.B. Weibel, W.R. DiLuzio, G.M. Whitesides, Microfabrication meets microbiology, Nature Reviews Microbiology 5 (2007) 209–218.
[40] E.L. Wollman, F. Jacob, W. Hayes, Conjugation and genetic recombination in Escherichia coli K-12, in: Genetic Mechanisms: Structure and Functions, Cold Spring Harbor Symposia on Quantitative Biology, vol. 21, 1956.
[41] T. Xu, S. Petridou, E.H. Lee, E.A. Roth, N.R. Vyavahare, J.J. Hickman, T. Boland, Construction of high-density bacterial colony arrays and patterns by the ink-jet method, Biotechnology and Bioengineering 85 (2004) 29–33.
[42] J. Yuan, K.A. Fahrner, H.C. Berg, Switching of the bacterial flagellar motor near zero load, Journal of Molecular Biology 390 (3) (2009) 394–400.

Pietro Lio' (pl219@cam.ac.uk) is a Senior Lecturer and a member of the Artificial Intelligence Division of the Computer Laboratory, University of Cambridge. He has an interdisciplinary approach to research and teaching due to the fact that he holds a Ph.D. in Complex Systems and Non Linear Dynamics (School of Informatics, dept of Engineering of the University of Firenze, Italy) and a Ph.D. in (Theoretical) Genetics (University of Pavia, Italy). He supervises 6 Ph.D. students and teaches the following courses: Bioinformatics (Computer Laboratory), Modeling in System Biology (System Biology Tripos, Dept of Biochemistry), Models and Methods in Genomics (MPHIL Comp Biology—Dept of Mathematics), 4G1 System Biology (Dept of Engineering). His main interests are in investigating relationships and effectiveness of different biosystems modeling methodologies (particularly multiscale approaches), modeling infectious diseases dynamics and Bioinspired communications and technology.

Sasitharan Balasubramaniam (sasib@tssg.org) received his Bachelor's (Electrical and Electronic Engineering) and Ph.D. degrees from the University of Queensland in 1998 and 2005, respectively, and Master's (Computer and Communication Engineering) degree in 1999 from the Queensland University of Technology. He joined the Telecommunication Software and Systems Group (TSSG), Waterford Institute of Technology (WIT), Ireland right after completion of his Ph.D. He is currently the manager for the Bio-inspired Network research unit at the TSSG. Sasitharan has worked in a number of Irish funded projects (e.g. Science Foundation Ireland, PRTLI) and EU projects. His research interests includes Bio-inspired Future Internet, as well as molecular communications.