

MoNaCo: FUNDAMENTALS OF MOLECULAR NANO-COMMUNICATION NETWORKS

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Unlike traditional technologies, molecular communication is a radically new paradigm, which demands novel solutions, including the identification of naturally existing molecular communication mechanisms, the establishment of the foundations of a molecular information theory, or the development of architectures and networking protocols for nanomachines.

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ABSTRACT

This article presents a branch of research where the use of molecules to encode and transmit information among nanoscale devices (nanomachines) is investigated as a bio-inspired viable solution to realize nano-communication networks. Unlike traditional technologies, molecular communication is a radically new paradigm, which demands novel solutions, including the identification of naturally existing molecular communication mechanisms, the establishment of the foundations of a molecular information theory, or the development of architectures and networking protocols for nanomachines. The tight connection of this cutting edge engineering research field with biology will ultimately enable both the bio-inspired study of molecular nanonetwork architectures and their realization with tools already available in nature. The testbed described in this article, which is based on a microfluidic device hosting intercommunicating populations of genetically engineered bacteria, is a clear example of this research direction.

INTRODUCTION

Nanotechnology is providing a new set of tools to the engineering community to control entities at the atomic and molecular scales. Foremost among these new capabilities are *nanomachines*, integrated functional devices which consist of nanoscale components, envisioned to accomplish tasks ranging from computing and data storing to sensing and actuation. Enabling nanomachines to communicate with each other and hence form *nanonetworks* will considerably expand their application domains [1].

Several communication paradigms can be considered for use in nanonetworks, but the focus of this work is on using *molecular communication*. In molecular communication, molecules are used to encode, transmit and receive information [2]. There are several different reasons for which using molecular communication is seen as being especially attractive:

- Molecular communications between nanoscale entities occur in nature. Examples include inter-cellular and inter-bacterial communica-

tion, and such natural phenomena offer a ready-made study ground both to model nanonetworks and to develop solutions;

- Nanonetworks can be built upon such naturally occurring phenomena with appropriate instrumentation and hence offer a faster engineering pathway to viable solutions;
- Several of the aforementioned applications require biocompatibility and hence necessitate properties that are readily offered by nanonetworks using molecular communication.

In this article, we take the position that not only molecular nanonetworks will have great relevance to biological physical systems, but also taking a bio-inspired approach to the design of nanonetworks is an optimal pathway to viable solutions. The outcome of this collaboration between disciplines will set the basis for future research and defines the first steps towards real implementable solutions. As an example, implantable nanomachines have been designed for medical applications to colonize and autonomously work inside the human body [3]. While their manufacturing is still in its infancy, the possibility of using biological entities as ready-made nanomachines, such as genetically engineered cells (e.g., bacteria), offers a possibly faster alternative to realize the envisioned applications.

The research on molecular nano-communication networks will make contributions along the following broad directions, reflected in the organization of the rest of the article. After a brief list of possible future applications of molecular nano-communication networks we outline the research on their information theoretical characterization. We introduce the investigation on the definition of suitable molecular communication protocols for the transmission of information. We describe a possible envisioned experimental platform. Finally, we conclude the article.

APPLICATIONS

There are a large number of applications envisioned for nanonetworks. We briefly present three main categories below, which capture the significance of the molecular nano-communication networks: biomedical, industrial, and surveillance applications.

A large number of applications of nanonetworks are in the *biomedical* field. Nanomachines can be deployed over (e.g., tattoo-like) or inside the human body (e.g., a pill or intramuscular injection) to monitor glucose, sodium, and cholesterol, to detect the presence of different infectious agents, or to identify specific types of cancer. Nanonetworks will also enable new smart drug delivery systems which combine the sensing capabilities of nanomachines with the abilities of nano-actuators to release specific drugs inside the body with great accuracy and in a timely manner.

The tools provided by nanotechnology can be used to monitor and control the formation of biofilms in several *industrial* applications. A biofilm is an aggregate of nano and micro-organisms in which cells adhere to each other and usually onto a surface. Biofilms can be used to clean residual waters coming from different manufacturing processes or organic waste. However, they can also be the tool for infectious diseases to spread through pipes and other liquid conducting mechanisms. In our vision, nanonetworks can be used to first detect the formation of biofilms and then to release specific chemical compounds to locally enhance or terminate their formation.

Nanotechnology is enabling the development of biological and chemical nanosensors which have an unprecedented sensing accuracy. Nanonetworks composed by several of these nanosensors will serve as a countermeasure for *surveillance* against nuclear, biological and chemical attacks at the nanoscale. For example, nanosensors can be used to detect chemical particles faster and in lower concentrations than conventional microsensors. Upon the detection of a toxic chemical compound, several nanomachines will transmit the information related to this event in a multihop way to a sink or command center. In addition, it will also be possible for the nanomachines to receive commands from the macroscale in order to, for example, change their behavior.

MOLECULAR INFORMATION THEORY

Molecular nanonetworks are directly inspired by communication networks among living entities already present in nature. Among others, we illustrate three examples, which differ in the way molecules propagate through diffusion from a network node (cells in the examples) to another.

Bacteria communication: Bacteria encode information, such as about their internal state, into the release of information molecules. These molecules propagate in the medium where the bacteria population grows and are sensed by other bacteria, which can decode the sent information.

Calcium signaling: A sensory cell, a neuron, or a cardiomyocyte releases or absorbs calcium molecules in response to various stimuli that open/close particular channels on the cell membrane. The molecular information in the variation of calcium ions concentration is propagated inside and outside the cell, causing a variation in the electrical charge of the cell membrane and, subsequently, the transduction of the information into an electrical signal.

Pheromone communication: Pheromones are a specific type of molecules released by plants, insects, and other animals that trigger specific behaviors among the receptor members of the same species. Pheromones of a particular type are released into the environment and propagate in the air until they are captured by the receptors of their same type.

The information theoretical research should capture all these three examples of molecular nano-communication networks by developing general mathematical models of the physics of communication by molecule exchange. These models should lead to the theoretical computation of the parameters used in communication theory, such as the attenuation and delay of the transmitted signal, the power and statistics of the noise sources, and, ultimately, the information capacity of the communication system.

MOLECULAR NANO-COMMUNICATION NETWORKS WITH TWO NODES

First, we focus on the most basic network composed only of two nanomachines or nodes. From the point of view of information theory, there are three main functional blocks constituting the network:

- The emission process (the *transmitter*)
- The propagation process (the *channel*)
- The reception process (the *receiver*)

as shown in Fig. 1.

We assume that the nanomachines are deployed in a space filled with a fluid medium, such as cellular cytoplasm or air, where molecules can propagate. In our article [4], we provide general models for the three functional blocks, which can be further tailored in order to capture the peculiarities of the aforementioned examples of nano-communication networks in nature. These general models are briefly described next.

The molecule emission process provides an output signal generated through the emission of molecules in a space according to a given input. Our emission process model from [4] is based on the modulation of the concentration of molecules according to a *molecule concentration rate*, which corresponds to the amount of molecules released in a time unit. The discrete nature of molecules affects the emission process by generating noise. Single molecules flowing out of the transmitter contribute to the emitted concentration rate at discrete time instants. These discrete time instants are not equally spaced, due to the random components in the motion of the molecules.

The propagation process provides the transport of the signal modulated at the emission process by means of a molecule *diffusion process*, which is defined as the movement of molecules in a fluid from an area of higher concentration to an area of lower concentration. As a result, the measured particle concentration suffers from two noise effects. The first effect is given by the quantization of the concentration measure by a discrete number of molecules present at the receiver. The second effect is given by fluctua-

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Nanomachines are simple entities which cannot perform complex tasks that render any traditional protocol abstractions untenable, but at the same time a new degree of freedom available in nanonetworks is that the channel is programmable, and hence active, at the nanoscale.

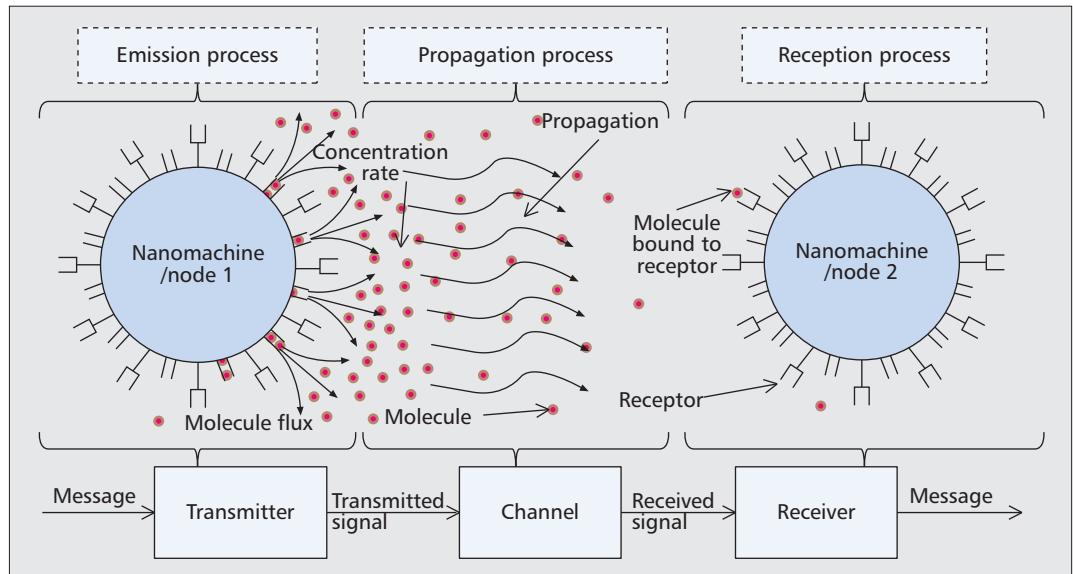


Figure 1. The scheme of a molecular nanonetwork with two nodes.

tions in the concentration measure due to single events of molecules entering/leaving the surroundings of the receiver.

The reception process provides the extraction of the information message from the received signal. In particular, in our preliminary work the receiver contains a set of N_R chemical receptors, and we focus on modeling the molecular receiver from the analysis of the ligand-binding process. Each chemical receptor can bind to molecules with a binding probability, or release previously bounded molecules with a release probability. The ratio between the number of bound chemical receptors and the total number of receptors is the output of the reception process and tends to be proportional to the input molecule concentration in the proximity of the receiver. The random fluctuations occurring in the chemical reactions underlying the ligand-receptor binding process generate noise in the reception process.

Each block in Fig. 1 has to be analytically modeled and investigated, resulting in their characterization in terms of *attenuation and delay*, noise statistics, and information *capacity*.

MOLECULAR NANO-COMMUNICATION NETWORKS WITH N NODES

We consider now a network composed by more than two nanomachines (Fig. 2). The presence of several nanomachines sharing the same medium affects the aforementioned processes and their models, which ultimately leads to differences in the information theoretical characterization of the emission, propagation, and reception of molecular information.

The emission process is affected by the presence of molecules released by other nodes. While the internal structure of a molecular emitter does not change to what we described above, its performance in terms of attenuation and delay varies. Different from classical communication, in molecular communication networks, the transmissions from other nodes *can physically*

block the transmission of a given nanomachine. While this may be a major problem in specific scenarios, we can also interpret this as an intrinsic mechanism in molecular networks to prevent the transmission of information in highly loaded networks.

The propagation model introduced above changes when multiple nodes share the same medium, since the molecules released by different nodes interact in the channel: they collide and change what would be their normal direction of propagation. This result is another major difference from interference in electromagnetic wireless networks. When multiple transmitters are present in the space, the high concentration of molecules raises the probability for them to collide. In this way, an additional emission process from a node can affect the propagation speed of the information coming from other nodes.

The receiver model we have described above does not fundamentally change for the case with multiple transmitting nodes. The main reason for this is that it is not based on a diffusion process, but on the ligand-binding theory. However, due to an expectedly increased number of molecules in the system, the noise affecting the reception of the molecular signal is expectedly larger.

INFORMATION CAPACITY

Given the peculiarities of molecular nanonetworks, the capacity analysis demands a complete rethinking of the classical information theory, stemming from the work by Shannon. The diffusion-based molecular channel is conceptually a *broadcast channel*. The molecules released in the medium linger in the channel for a long time. We refer to this property as the *memory* of the molecular channel. Furthermore, the concept of *interference* in the molecular network is radically different from that in electromagnetic or acoustic networks. First, there is a high degree of randomness in the behavior of a biological entity such as an engineered bacterium, making reli-

able communication between two entities nearly impossible. Second, molecular signal transmission and reception processes tend to be very slow relative to traditional communication technologies, limiting the frequency of the channel use.

One approach to capacity analysis is to consider the case where the molecules produced by the transmitter node would induce different levels of concentration at the receiver [5]. Four factors would contribute to the uncertainty of the molecular communication in such a setup:

- Uncertainty in the molecular output of a transmitter node
- Uncertainty induced by the diffusion channel
- The probabilistic nature of the number of activated chemical receptors at the receiver
- The heterogeneous behavior of the biological entities residing in a receiver node

Incorporating all these uncertainties, the theoretical limits of the information transfer rate in such a setup can be studied vs. the design parameters (e.g., number of biological entities per node, number of receptors per biological entity, and maximum power).

To facilitate reliable communication among nodes, an error control mechanism is needed. Since nanomachines are limited in their processing capabilities, coding mechanisms mostly take the form of repetition coding for a molecular channel. However, this results in a substantial drop in the achieved rate. As a better alternative, we propose multi-molecule transmission as a simple coding strategy. Suppose that in the network model described above, node S uses two types of molecules, m_1 and m_2 , for message transmission. One simple operation we may assume for every relay node R_i is that it can form the *state of aggregated belief*. That is, upon reception of molecules, the relay node senses the concentration of both molecules and forms a belief (estimate) about the concentrations. Following this process, if the probability of error for receiving each type of molecule was p_1 , now our system works with the probability of error $p_2 < p_1$.

STUDY OF MOLECULAR NANO-COMMUNICATION PROTOCOLS

Traditional notions for the design of communication protocols cannot be reused in molecular nano-communication networks due to their unique characteristics stemming from the diffusion-based propagation of molecules. In traditional protocol design, the end systems and intermediate entities are assumed to be able to perform complex tasks, whereas the channel itself is assumed to be simplistic. Nanomachines are simple entities which cannot perform complex tasks, rendering any traditional protocol abstractions untenable, but at the same time a new degree of freedom available in nanonetworks is that the channel is programmable, and hence active, at the nanoscale. It is this degree of freedom that we exercise in constructing protocol abstractions for nanonetworks. We consider a broad *nano-active channel* paradigm within which we design and develop the proposed protocol solutions.

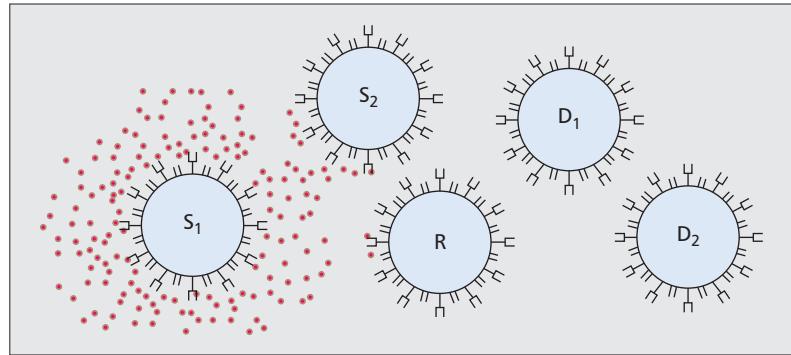


Figure 2. A nanonetwork composed of five nodes.

We approach the task of developing the paradigm of nano-active channel protocols by breaking down the solution into three distinct components:

- We first explore communication *protocol primitives* that can be derived from the capabilities and characteristics of the nanomachines.
- We then identify and develop *techniques* for nanoscale communication that should be used as guiding concepts along with the primitives in designing higher order functionalities.
- We design communication *protocol abstractions* that combine a set of primitives, in a particular order and with the constraints of the guiding techniques, for the realization of more sophisticated functionalities.

PROTOCOL PRIMITIVES FOR NANOSCALE COMMUNICATION

Our goals in identifying primitives is to arrive at a set of core building blocks that satisfy two requirements:

- They are *achievable* building blocks given the target operating environment, and are preferably derived from the entities in the environment as is.
- They can be used to achieve complex (and composite) protocol abstractions.

We now briefly discuss a few generic primitives for a nanoscale environment.

In **Send**, molecules are externally introduced into the system. The input to this communication primitive is the signal to be transmitted. This can be encoded into either the desired concentration of a type of signal molecule in the fluid medium or physical triggers that stimulate the release of the signal molecules from the transmitting entity. The output of this primitive is the effective modulated concentration of the signal molecules in the fluid medium, which causes the reception of the desired signal by the receiving entity. In **Block**, molecules are absorbed and removed from the system, possibly through the intervention of a nanomachine. **Forward** will amplify and forward a particular received signal. **Transform** is a communication primitive that will convert one signal into another signal. **Insert-Identifier** allows some method of including identifiers in the information carrier. For instance, this can be achieved using biological tags, which are biological entities widely used in selective reaction between the informa-

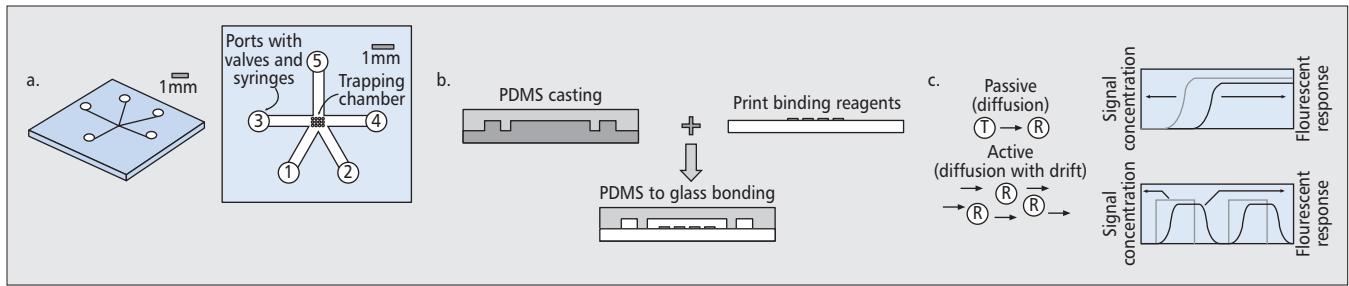


Figure 3. a) A multilayer microfluidic device that can be designed to allow input/output ports to deliver reagents, waste, and cells to a trapping chamber; b) fabrication steps of the device; and c) stimulation and measurement of the response of the cells to both constant and time varying molecular signaling conditions in either a passive or active diffusion.

tion carrier and the receiver. **Conditional-Receive** allows a nanomachine to receive information from a specific subset of information carriers. **Logical-Combine** is a communication primitive that allows a nanomachine to compute the logical functions of the inputs received from multiple transmitter nanomachines. This can be realized using approaches in recent research as described in [6].

TECHNIQUES FOR PROTOCOL DESIGN IN MOLECULAR NANONETWORKS

While primitives are grounded in what is practically possible/controllable for communication in the target nanoscale environment, we now present some insights into guiding techniques that need to be used in tandem with the primitives while constructing higher order communication abstractions. As with primitives, we present these as exemplifying techniques and will consider the universality of such techniques as part of the molecular nano-communication network research.

Tandem activation involves multiple transmitter nanomachines that are used simultaneously in a cooperative manner. When multiple transmissions happen concurrently in a shared medium, the signals naturally combine in the channel after incurring channel impairments. When the transmissions are uncoordinated they lead to interference at the receiver nanomachines. However, by coordination of transmitter nanomachines, the detrimental effects of interference can be overcome, and the network performance can be improved. Recent research illustrates how logical functions of inputs can be realized using molecular reaction processes [6].

Gap intervals is a communication technique inspired by the timing channel in classical communications, which is based on the emission of concentration pulses. This allows maintaining the number of molecules in the fluid medium at a low level. The first pulse denotes the start of the information unit, and the second concentration pulse denotes the end of the unit. The time interval or *gap interval* between the start and stop concentration pulses will convey the information.

Selective attachment is based on the capability of nanomachines to selectively/preferentially attach to certain other molecular machines. This attachment can be triggered using a variety of

molecular processes. For instance, the *E. coli* bacteria is attracted by certain chemicals called attractants and swim toward high concentrations of attractants using flagella. Recent works illustrate how *E. coli* can be genetically modified to be attracted to a specific chemical attractant only [7]. This allows selective reception of information molecules.

PROTOCOL ABSTRACTIONS FOR MOLECULAR NANONETWORKS

A final goal beyond nano-communication primitives and techniques is to combine the primitives for constructing nanonetwork protocol abstractions that can provide complex communication functionalities. We present examples of such abstractions next.

Assured state transfer is responsible for reliably sending state information through signaling molecules from source to destination. The particle primitives should be executed in the following sequence:

- State signal molecules (signal 1) are released from a source (send).
- Nanomachines that are capable of forwarding the molecules propagate the signal molecules to the destination (forward).
- The destination absorbs the signal 1 molecules and releases a different type of signal (signal 2) back into the medium (transform).
- Signal 2 molecules are forwarded back toward the source (forward).
- Signal 2 acts as a trigger for the nanomachines responsible for blocking signal 1 molecules from propagating in the medium.

This ensures that no more signal 1 molecules are sent toward the destination, and the state is reliably conveyed.

Causal delivery is a protocol abstraction where different biological processes need to be executed in a particular order. Such an abstraction would be critical for an environment where directives are being sent to both different nanosensors/actuators at the same time. For example, an application may require two signals (signals A and B) to be processed in order. To achieve this, two primitives, forward and block, can be used in the following way. The forward signal primitive is applied on signal A, and the signal block primitive is applied on signal B for a period of time to allow A to be delivered, at which point the block primitive is changed to a forward primitive.

Directed delivery is a protocol abstraction that enables communication of information from a specific nanomachine to another specific nanomachine in a network of nanomachines. This problem has similarities to the addressing problem in a large network of nodes. This can be achieved by leveraging the selective attachment technique described previously. To achieve this, different chemical or biochemical attractants that can be applied to a given environment must be identified and allocated intelligently to different transmitter/receiver nanomachines in the same environment.

EXPERIMENTAL VALIDATION OF MOLECULAR COMMUNICATION

The design and implementation of molecular nanonetworks requires a concerted effort ranging from modeling the system to experimentation of simple, tractable molecular cell networks. We envision the development of an experimental platform for validating molecular nano-communication networks by using a well-described molecular cellular communication system in bacteria called *quorum sensing* (QS). What is learned regarding the limits of molecular signaling in this simple system will serve as a proof of concept for theoretical models and can be applied to more complex molecular nano-communication systems.

COMMUNICATION AMONG BACTERIA

QS is an example of communication by which bacteria synchronize gene expression on a population-wide scale [8]. In QS, each bacterium of a population emits a particular kind of molecules (autoinducers) in the medium where the population resides. If the concentration of autoinducers in the medium is over a predetermined threshold, this triggers regulatory changes within the bacterium that activate the expression of gene encoding factors. This concentration threshold can generally be reached only if the number of bacteria in a population is significant and thus the overall rate of emission of autoinducers is sufficient for their accumulation in the medium. QS systems provide an apt environment that captures all of the elements of molecular communication systems and hence can be used to validate/refine the theoretical developments of molecular nano-communication networks. In particular, we focus on the *Vibrio fischeri* (*V. fischeri*) paradigm as an example model nano-communication network.

In *V. fischeri*, the expression of the QS-controlled genes is regulated in a cell-density-dependent fashion through the synthesis of the signal molecule (3-oxo-C₆-HSL), abbreviated C₆-HSL here. The LuxI enzyme synthesizes the C₆-HSL signal, which freely diffuses through the bacterial membrane and is emitted into the medium surrounding the bacterial cells. Upon binding of the signal to its receptor, the C₆-HSL/LuxR complex acts as a transcriptional activator binding to DNA in the *lux* operon promoter region that activates the expression of the *luxI* gene. This translates to positive feedback loops amplifying the signal production in response to reception.

In a population of *V. fischeri*, bacterial cells are genetically identical; each cell serves as both a transmitter of the signal and a receiver. However, *V. fischeri* can be *engineered* to serve only as transmitters or receivers.

GENETIC ENGINEERING OF A SIMPLIFIED NANONETWORK

We envision an experimental platform composed of a microfluidic device in which we can incubate bacteria that are genetically engineered to encode a well described molecular signaling circuit. Specifically, the model organism *E. coli* can be engineered to encode components of a QS molecular communication network from the bacterium *Vibrio fischeri*. This synthetic biology approach will recreate, in *E. coli*, a complex cellular behavior that can be perturbed and manipulated to understand the networking fundamental variables.

The envisioned design of the experimental platform consists of different populations of *E. coli* cells: transmitters (T) and receivers (R). In this platform, the transmitter cells contain the *V. fischeri luxI* gene under control of a non-native (IPTG-inducible) promoter that allows *luxI* expression to be controlled experimentally. Alternatively, transmitter cells can also be designed with the *luxI* gene under control of the *V. fischeri luxI* promoter and thus maintaining the auto-feedback loop. Like the receiver cells, the transmitters may contain the *luxR* gene under control of a promoter so that the LuxR receptor is constitutively made. Receiver cells contain both the *gfp* gene for GFP protein under control the *V. fischeri luxI* operon promoter and the *luxR* gene.

EXPERIMENTAL PLATFORM DESIGN

We utilize a microfluidic device to host populations of genetically engineered bacteria by providing the necessary space, nutrients, and a common medium where information molecules can diffuse and propagate between different populations (Fig. 3). This will enable the direct testing of a nanonetwork, and dynamically measuring communication parameters such as delay vs. frequency, amplitude vs. frequency, noise, and interference.

Microfluidic devices have been widely used for biological analysis as they provide benefits, including small device sizes, low reagent volumes, and the possibility for parallel processing. Previous researchers have demonstrated the utility of microfluidic devices for studying bacteria communication. Danino *et al.* developed a device for measuring spontaneously generated synchronous fluorescence oscillations in populations of cell colonies [9]. Fernandes *et al.* utilized biological nanofactories and electrodeposited chitosan to spatially capture and alter the response of QS bacteria within a microfluidic device [10]. These technologies show exciting emerging applications of genetically engineered QS bacteria in a controllable microenvironment; we develop a platform to explore the limits of this communication in a high-throughput, time- and space-varying manner.

We envision the development of an experimental platform for validating molecular nano-communication networks by using a well described molecular cellular communication system in bacteria called quorum sensing.

We have taken the position that not only will molecular nanonetworks have great relevance to biological physical systems, but also taking a bio-inspired approach to the design of nanonetworks is an optimal pathway to viable solutions.

CONCLUDING REMARKS

In this article, we have discussed the broad areas in which the research on molecular nano-communication networks will make contributions in the near future. The identification of existing molecular communication mechanisms, the establishment of the foundations of molecular information theory, the development of architectures and networking protocols for nanomachines, and the engineering of reliable experimental platforms are essential steps toward the objective of establishing the theoretical foundations of molecular nanonetworks and to pave the way for this new networking paradigm. In this article, we have taken the position that not only will molecular nanonetworks have great relevance to biological physical systems, but also taking a bio-inspired approach to the design of nanonetworks is an optimal pathway to viable solutions. The outcome of this collaboration between disciplines will set the basis for future research and define the first steps toward real implementable solutions.

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BIOGRAPHIES

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