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Protocols for Molecular Communication Nanonetworks

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Doctor of Philosophy

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Declaration

I hereby certify that this material, which I now submit for assessment on the programme of study leading to the award of Doctor of Philosophy, is entirely my own work and has not been taken from the work of others save to the extent that such work has been cited and acknowledged within the text of my work.

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Date: October 2, 2013

Abstract

Advances in nanobioscience and nanomaterials have resulted in biological nanomachines, or *bionanomachines*, that can perform tasks at the molecular scale and promise novel applications in the areas of medical, biological and nano science. A key enabler for these applications is the creation of nanoscale networks or *nanonetworks* which will facilitate communication and collaboration between bionanomachines and communication with external networks. However, the creation of a biological nanonetwork using conventional electromagnetic communication technology is constrained by the physical scale, biological compatibility factors, and the computational limitations of the biological nanomachines.

Inspired by natural biological processes, *molecular communication* is an emerging communication paradigm that uses biological molecules to encode and transmit information. The current research in this domain has concentrated predominantly on the physical mechanisms and channel models involved in encoding and transporting molecular encoded information in biological environments. This thesis extends this work by developing communication protocols for molecular communication nanonetworks. More specifically, this research maps existing networking concepts such as addressing, routing and message scheduling to biological processes and shows how these processes can be integrated with different modes of molecular communication. Components from various layers of a communication protocol stack are matched to suitable molecular computing mechanisms. Nucleic acid-based molecular computing solutions are used to design and simulate protocol components for information encoding and addressing of biological molecules whereas enzyme-based molecular computing solutions are utilised for routing and switching protocol functions. The performance of neuronal nanonetworks is investigated taking into account how neuron cell characteristics affect message delivery. This includes a genetic algorithm-based transmission scheduling approach to ensure that signals initiated by multiple devices will successfully reach the receiver with minimum interference. The reliability and delay characteristics for multi-hop, virus-based nanonetworks is also investigated and a probability model is developed. This is used to evaluate different topology designs, taking into account the physiochemical and biological characteristics of virus particles.

The final simulation results and analysis models characterise several approaches to nanonetworking using different modes of molecular communication and provides the capability for accurately designing molecular communication nanoneworks. It is expected that this work will make a significant contribution to the *in-silico* design and development of future nanoneworks.

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Contents

Chapter 1

Introduction

1.1 Background and Motivation

This section describes the background work and motivating factors that form the basis for this thesis. We discuss the advent of nanomachines and, supported by advances in synthetic biology, the concept of biological nanorobots and nanomachines (*bionanomachines*). We then describe the future applications and communication requirements predicted for bionanomachines, focussing particularly on biological and biomedical applications. Finally, the current challenges for bionanodevices and nanonetworks are described.

1.1.1 Biological Nanomachines and Applications

The term *nanodevice* or *nanomachine* can be applied to a device whose constituent parts are of the order of nanometers ($10^{-9}m$) in size and which has the ability to perform tasks at the molecular level. One of the earliest references to nanomachines in a biological setting is attributed to Feynman in 1959 [1] in which he predicted using nanodevices as "mechanical surgeon(s)", allowing patients to essentially "swallow the surgeon". Since then, the extensive growth of nanotechnology research has produced several sub-domains including a particular focus on the application of nanomachines in a biomedical (nanomedicine) and biological (nanobiotechnology) setting, in which many of the future applications are forecast.

Nanomachines can be created using one of three approaches: (1) top-down; (2) bottom-up; and (3) bio-hybrid [5]. Top-down nanomachines are developed by scaling down existing microelectronic components, without atomic level manipulation. The bottom-up approach creates nanomachines from molecular components either manually or through self-assembly, for example DNA self-assembling nanomachines [46]. The bio-hybrid approach is based on the use of existing biological systems and mechanisms, such as molecular motors, as components in synthetic engineered bionanomachines [45]. Furthermore, the bionanomachines can take the form of engineered biological cells where some of the cell functions have been re-purposed to perform a specific nanoscale task such as sensing or actuation. The bio-hybrid approach provides advantages particularly to nanomedicine and nanobiotechnology applications due to their

inherent compatibility with other biological processes and reduced energy requirements.

There is a large number of potential applications for bionanomachines. A good example is in the detection and monitoring of cholesterol or disease precursors in tissues to provide proactive medical diagnosis [2]. Machines acting in a drug delivery capacity could deliver precise quantities of chemicals to specific cells in collaboration with other peer machines [3]. In doing so, adverse side effects inherent in conventional drug delivery mechanisms could be avoided. Machines acting as surgical assistants can find, isolate and highlight damaged or malignant cells while protecting normal tissue [51][52][50][53]. The distinct advantage of their size gives nanomachines the ability to work as assemblers, accurately building molecular scale components. The feasibility of such novel applications is reliant on autonomous, programmable machines and these applications are being approached rapidly. Several examples of bionanomachines have been demonstrated experimentally [9][10]. However, currently these applications use autonomous nanomachines that do not communicate. This restricts potential applications and, as Feynman also pointed out, many future applications will have the following requirement: "the information has to be fed out". Moreover, information must also be shared between peer biological nanomachines in order to collaborate effectively and achieve their full potential. Bionanomachines, due to their size, will not have the ability to perform complex operations in isolation. For example, the drug delivery application predicted in [3] requires communication and coordination of several bionanomachines in order to perform the overall task. Furthermore, bionanomachines operating in a sensory capacity will have a very small operating range and some applications will involve the deployment of large volumes of bionanodevices. Communication mechanisms will enable the sensory data to be shared and transmitted out of the environment. This suggests that nanoscale communication mechanisms or networks (*nanonetworks*) are vital at this scale, facilitating inter-machine communication and communication with higher level and external processes and networks.

1.1.2 Communication between Bionanomachines

Communication between bionanomachines is critical to providing the future applications described in the previous section. Communication between bionanomachines can be catagorised as "wet" or "dry" [11]. The dry approach is characterised by nanoscale silicon and carbon components [48], often combined with electromagnetic or optical-based communication channels. This approach relies on the ever increasing sophistication in silicon manufacturing to scale down micro-scale components to the nanoscale. For example, wireless antennae have been produced at this scale [4] however, their use for bionanomachine communication is difficult predominantly due to power requirements, size constraints and compatibility issues with biological environments [5]. "Wet" techniques, on the other hand, generally use biological components that are compatible with the aqueous environment prevalent in a biological setting such as living tissues. Living organisms already use communication mechanisms at the nanoscale: biological cells use chemical and biological signals to control internal functions and to communicate externally with other cells. This mode of communication has inspired the emergence of *molecular communication* [7], a communica-

tion paradigm which uses biological molecules and mechanisms to encode and transmit information. Realising communication between bionanomachines using molecular communication has the advantage of environment compatibility and offers the possibility of re-purposing existing biological communication mechanisms. There are several molecular communication modalities, some of which will be discussed in section 2.1. The properties of molecular communication channels at the nanoscale contrast significantly with conventional communication channels as follows [7]:

- Signals in molecular communication are often governed by molecular diffusion. This is extremely slow relative to conventional networks (speed of light).
- Channel conditions such as pH, temperature and diffusion coefficient can significantly affect communication.
- Noise model in molecular communication is based on chemical and particle interference, not electromagnetic interference.
- Transmission to the channel is low energy (when using biological mechanisms relative to conventional wireless communication).
- Information is encoded in molecule modulation. Conventional networks encode by modulating electromagnetic signals.

Molecular communication can provide a suitable new communication paradigm for biological nanonetworks and can overcome the restrictions inherent in applying traditional communication methods. However, in addition to the constraints of nanomachines relating to size, manufacture and deployment, researchers must also take into account the characteristics of molecular communication as detailed in the above list. Furthermore, researchers must investigate ways in which bionanomachines can integrate with and use molecular communication modes.

1.1.3 Biological Nanonetworks Requirements

In order to participate in molecular communication nanonetworks, bionanomachines must have the ability to modulate message molecules using either concentration encoding or molecular encoding. In the case of concentration encoding, bionanomachines encode information into a temporal molecule concentration sequence. Receiving nanomachines can then decode the molecular concentration sequences. In molecular encoding, information is encoded into the molecule itself, such as modulating the physical shape or chemical structure of the molecule (e.g. encoding data into the nucleotide sequence of a DNA molecule). These encoding techniques must be decodable at the intended receiver. This implies that the bionanomachines must have access to the computational capability to encode, send and receive information via molecular communication in order to participate in and use molecular communication nanonetworks: they require the implementation of communication protocols.

Similar to molecular communication, *Synthetic Biology* seeks to reuse and re-engineer existing genetic, enzymatic and cellular systems to design and create computational components. Recent advances in this area have been used to create automatons [12] and logic circuits using cell based systems, essentially

re-purposing the cell as a computational component. In fact, the standard component approach that has proved successful in electronic engineering is now being applied in synthetic biology [54] to promote a modular approach to creating useful future applications.

This work takes a similar approach to communication protocol development, using existing molecular communication modes for the physical communication layer, and a synthetic biomolecular computation approach to provide data link, network and transport layer functions to create communication protocols for molecular communication nanonetworks. This poses several challenges that must be met and are discussed in the next section.

1.1.4 Challenges

The previous sections describe the progress and potential of biological nanonetworks. However, the domain is still in its infancy with many outstanding challenges. This work proposes that specific communication protocols and interfaces are vital to the success of molecular communication nanonetworks. However, there are significant challenges to the design and development of biological nanonetworks and it is these challenges that are addressed in this research. The challenges are presented in the context of a molecular communication-based physical layer, specifically for bionanomachines.

C1: Communication Protocols

Just as layered communication protocol stacks form the basis of conventional data networks, so the development of a suitable communication protocol stack for molecular communication based nanonetworks will provide the modularisation to facilitate the design and creation of complex nanonetwork architectures. Molecular communication protocol stacks will need to be adaptable to the large spectrum of physical layer modalities possible for molecular communication physical layer. Furthermore, while the conceptual design of a layers communication model (datalink, network, application) can be inspired from existing models (OSI/TCP), the actual implementation of a protocol stack must be informed by the size, environment and capability constraints at this scale, and also by the availability and capability of the synthetic components used to implement each layer of the stack.

C2: Compatibility and Integration

We predict the fusion of synthetic molecular computation and molecular communication will facilitate and expedite the nanonetwork applications of the future. The challenge of combining both domains for communication protocols has two key areas. First, in the external integration of a synthetic engineered protocol stack to a molecular communication physical layer and to potential applications at the application layer. Secondly, in the interconnection between layers in the protocol stack. Compared to conventional data networks, the homogeneous nature of electromagnetic based communication networks makes it relatively easy to define interfaces and inter-connect functions of protocol stacks. However, the heterogeneous nature and specificity of biological processes pose a significant challenge. For example, engineering an interface for a chemical enzyme based

logic circuit to control an existing and completely separate calcium signalling process in a biological cell is difficult. Taking a bio-hybrid approach does have the advantage of bio-compatibility, but significant constraints will need to be addressed in the integration of distinct biological processes. One approach to this would be to map existing protocol operations such as addressing, routing and transport, to existing integrated cellular and biological signalling pathways, essentially re-purposing existing pathways in the cell and mapping different stages of the pathway to communication protocol stack functions.

C3: Biological Component Characteristics

The creation of molecular communication nanonetworks is reliant on the repurposing of existing biological mechanisms and processes. The characteristics of these processes will pose significant challenges to nanonetwork architectures and topologies. For example, biological particles such as viruses and DNA have a decay rate or half life which imposes an effective communication distance based on the communication channel characteristics. This requires a network architecture that contains replicating nodes for communication beyond the effective distance. Furthermore, biological signalling mechanisms, such as calcium ion signalling, have a significant refractory time after they are fired, during which they cannot generate or forward signals. This is a constraint in multiple access channels that consist of several transmitting nodes and must inform the architecture and topology design of nanonetworks.

1.2 Research Scope of the Thesis

The scope of the research presented in this thesis is discussed in this section. Section 1.2.1 presents the limitations of current nanonetworks, which are the focus of this thesis. Section 1.2.2 discusses the main objectives of the research, represented in the form of research questions.

1.2.1 Barriers to Molecular Communication Nanonetworks

The range of possible functions that an individual bionanomachine can perform is limited. Therefore, a performance improvement may be achieved by increasing the communication capabilities of these machines in order to provide for the collaborative applications previously discussed in section 1.1. The improvement requires further development of interfaces and protocols for nanonetworks. This part of the thesis considers the following limitations:

Lack of Communication Protocols for Bionanomachines There is a significant body of research into different modes of molecular communication. Similarly there has also been research into the modelling and analysis of molecular communication channels, relating to information theory and channel noise, which will be discussed in section 2.1.3. However, the solutions are predominantly concerned with the physical mechanisms used, and concentrate on the channel performance in relay channels for communication between two devices. In order to address the communication requirements of future bionanomachines,

this work needs to be extended into the development of suitable communication protocols for molecular communication nanonetworks.

Nanoscale protocol implementation Bionanomachines, due to their size, are functionally constrained both mechanically and computationally. Currently it is envisaged that a single bionanomachine will perform simple tasks and will not have the diversity of purpose provided in micro scale counterparts. Also, transmission and device speeds are extremely slow in comparison with conventional data networks and devices. Many of biological mechanisms found in biological cells provide interfacing and switching functions that could be re-purposed for molecular communication nanonetworks. These biochemical reactions perform much slower than conventional network functions such as switching and routing. For example, the behaviour of biological components as digital logic functions lack the speed and uniformity of conventional electronic components. The near instantaneous square wave response, typical in silicon digital electronic circuits, contrasts significantly with the slower, more sinusoidal response in biomolecular mechanisms used to implement protocol logic. This needs to be characterised.

Heterogeneity A diverse set of environments exist in which bionanoneworks will be deployed. Similarly, a diverse set of molecular communication modes exist at the physical layer. Taking the example of possible biomedical environments, bionanomachines may be deployed both inside and outside the cells. They may be stationary in tissues or mobile in the bloodstream. Various types of tissue can have diverse characteristics. Aqueous based environments will have diffusion constants of approximately $3.6\mu m^2 s^{-1}$ while tightly packed tissue structures using mechanical contact communication, will be constrained by gap junction permeabilities. In relation to molecular communication, many modes of molecular communication exist with contrasting characteristics and will be discussed in section 2.1.

Noise in Protocol Components As will be discussed in section 2.1.3, the noise model in a biological setting can differ significantly from conventional electromagnetic noise models and is currently being explored by several researchers. Noise also affects the operation of protocol functions implemented using biological components. For example, the concentration of a biological species used to represent a logic state can be the subject of interference from another separate biological/chemical process, the biological equivalent of crosstalk in wired electromagnet channels. Also, the physiochemical properties such as temperature and diffusion coefficient can also affect biological species concentrations.

1.2.2 Research Objectives

Research presented in the thesis explores the development of communication protocols that address the barriers presented in the previous section and the challenges presented in Section 1.1.4. This has resulted in the following three overarching research themes:

- Mapping conventional protocols to molecular communication protocols.

- Utilising biological cells as a nanonetwork communication platform for protocol development.
- Analysis of scheduling and routing in molecular communication nanonetworks.

These research themes are addressed in the following three sets of specific research questions (RQs):

Mapping conventional protocols to molecular communication protocols

RQ1.1 *How can existing communication protocol concepts be applied to bionanomachines in order to successfully use molecular communication nanonetworks for inter-nanomachine communication (Challenge C1)?*
RQ1.2 *How can DNA computing techniques be utilised to develop information encoding, addressing, and forward error correction (FEC) protocol functions in molecular communication nanonetworks (Challenge C1)?*
RQ1.3 *What biological enzyme computing techniques can be used to develop transmission and error recovery protocol functions for molecular communication nanonetworks (Challenge C1)?*

Utilising biological cells as a nanonetwork communication platform for protocol development

RQ2.1 *How can Mitogen Activated Protein Kinase (MAPK) enzyme pathways be utilised to create cell-based computational logic for communication protocol components in biological cells (Challenge C2)?*
RQ2.2 *In what way can the RNA interference (RNAi) pathway be used to create molecular communication protocol components in biological cells (Challenge C2)?*
RQ2.3 *In what way can nucleic aptamers be used as protocol adapters in order to integrate different biological protocol components in a cell (Challenge C2)?*

Analysis of scheduling and routing in molecular communication nanonetworks

RQ3.1 *Can an effective transmission scheduling protocol be developed to address the refractory time constraints of neuronal based multiple access molecular communication nanonetworks (Challenge C3)?*
RQ3.2 *What is a suitable probability model for use in the analysis of delay and reliability of end-to-end communication in multi-path virus-based nanonetworks (Challenge C3)?*
RQ3.3 *How can multi-path network topologies be used to maximise reliability and minimise transmission delay in virus-based multi-hop nanonetworks (Challenge C3)?*

1.3 The Approach

In this section we describe the research approach used to answer the research questions from the previous section. We outline the approach used in each general research theme.

1.3.1 RQ1.*: Mapping conventional protocols to molecular communication protocols

Chapter 2 will present an overview of current solutions in molecular communication, which are organised into two separate categories (wired and wireless). Conventional communication protocol stacks adhere predominantly to the standard layered approach put forward in the OSI [56] and TCP/IP [57] references. Our approach investigates the application of layered communication stack model in a biological nanonetwork setting. This approach assumes that molecular communication nanonetworks will employ a stacked network architecture to support the heterogeneity that is inherent in molecular communication physical layers. We investigate the implementation of protocol components using biological computation techniques and components from synthetic and molecular biology research, re-using existing models and components from this domain to design and simulate nanonetwork transceivers that can be integrated with several molecular communication physical channels.

Furthermore, we isolate particular protocol functions such as addressing and error correction and map them to the most suitable biological techniques such as DNA-based and enzyme-based computation.

1.3.2 RQ2.*: Utilising biological cells as a nanonetwork communication platform for protocol development

The biological cell has already been used for sensing and signalling by re-engineering internal signalling and regulatory pathways [92][93][98]. Building on the results from the previous research questions using biological computation, we consider adapting the genomic and enzymatic functions of the biological cell to create a generalised communication platform that can be used by bionanomachines to communicate and integrate with nanonetworks. From a networking perspective, we look to utilise the biological cell as a network transceiver for biological nanomachines. We take cues from existing system biology models to develop biological protocol component models, using published and curated models from System Biology to design cell-based protocol components. For example, we reference and adapt curated System Biology Markup Language models (SBML [91][96]) for cell-based calcium ion signalling [95] to create the models that form the basis for our protocol simulations. Similarly, the molecular communication channel models are informed from published, accepted dynamic models that describe the underlying physiochemical processes. We also access published research in nucleic acid-based computation to develop genetic pathway-based protocols in the cell. We use industry standard tools such as Cell Designer [97] and Matlab [71] to simulate our protocol models. This approach is applied to several molecular communication modes and computational techniques during the lifetime of this research.

Research Question	Challenge	Achievement	Publication	Appx.
RQ1.1	C1	A1	ICST NanoNet, 2007	1
		A2	ICST Bionetics 2008	2
RQ1.2	C1	A3	ICST NanoNet 2009	3
RQ1.3	C1	A4	ICST NanoNet, 2007	1
RQ2.1	C2	A5	ICST NanoNet 2009	3
		A6	NanoComNet 2010	4
RQ2.2	C2	A7	NanoComNet 2010	4
RQ2.3	C2	A8	NanoComNet 2010	4
RQ3.1	C3	A9	IEEE MoNaCom 2011	5
		A10	NanoComNet 2011	6
RQ3.2	C3	A11	IEEE MoNaCom 2013 (Accepted)	7
RQ3.3	C3	A12	IEEE NanoTechnology 2013 (Accepted)	8

Table 1.1: Research Contributions

1.3.3 RQ3.*: Analysis of scheduling and routing in molecular communication nanonetworks

We develop analytical and simulation models for two types of molecular communication nanonetworks (wireline network using neurons and wireless using virus particles)and simulate the performance of different architectures and topologies while referencing the results from the previous research questions. The models and parameters we use are informed by relevant research output from experimental and system biology. Through simulation we analyse the effects of various physiochemical characteristics on the reliability, transmission delay, and message loss in the various topologies.

1.4 Contribution to the Domain

We believe this thesis makes significant contribution to the domain of bionanotechnology. This section summarises the main achievements contained in the thesis. Table 1.1 maps the achievements to the corresponding research questions and to the peer reviewed research papers that provide the output of this thesis.

1.4.1 RQ1.* : Mapping conventional protocols to molecular communication protocols

A1: We have developed and simulated a general molecular-based communication protocol that uses an explicit acknowledgement protocol for reliable communication between nanomachines [61]. The protocol design is based on natural compartmentalisation and scaffolding techniques, using a recurrent enzyme-based logic circuit.[68]

A2: We defined a general protocol stack for molecular communication between bio-nanomachines. In doing so we identify key considerations and suitable bio-computation components to implement the respective protocol components. We identify nucleic acid-based computation as suitable for molecular encoding and enzyme-based computation for switching and routing protocol functions [59].

A3: We proposed a design for specific communication protocol stack components for biomolecule address encoding, decoding, and error correction using DNA-based computation techniques. Our solution applies existing techniques used in DNA-based automata and computation [12][64].

A4: We define an error-recovery process using a chemical state machine based on an enzymatic nano-logic circuit. We identified and demonstrated, through simulation, the challenges for molecular communication protocols due to chemical instability in protocol logic implemented in enzymatic circuits [61].

1.4.2 RQ2.* : Utilising biological cells as a nanonetwork communication platform for protocol development

A5: We designed a link switching layer for molecular communication using the output of the enzyme-based circuit. This provides the capability to switch biomolecules between molecular communication interfaces (in this case, by modulating the permeability of cell gap junctions) in a synthetic cell [64].

A6: We have defined, modelled and simulated a communication protocol solution using MAPK cell-based enzyme signalling pathways for computing. This solution was coupled with a calcium ion signalling physical layer, encoding through both amplitude and frequency modulation. Furthermore, we design and simulate a simple addressing protocol function for calcium ion molecular communication using amplitude encoding [60].

A7: We designed and evaluated through simulation, a communication protocol solution using RNA interference (RNAi)-based computing circuits and couple this with a virus particle molecular communication physical layer. This uses a RNAi logic circuit to control the packing and release of virus particles from an engineered cell [60].

A8: We proposed the use of aptamers as a modular solution to integrate RNAi-based application layer component with the occurrence of a specific chemical event. This solution allows RNAi-based protocol functions to detect the occurrence of a particular chemical event based on aptamer design [60].

1.4.3 RQ3.* : Analysis of scheduling and routing in molecular communication nanonetworks

A9: We modelled and simulated several topologies for backbone neuron networks and analysed the message blocking rate performance for each topology, using a varying number of devices and transmission rates. This allowed us to

determine how the patterns of transmitted events can be affected by the topological shapes of neuron networks [63].

A10: We used a genetic algorithm-based approach to create a transmission schedule to improve reliability and reduce message blocking of neuronal backbone networks. Analysis of the scheduling algorithm through simulation demonstrates that signals initiated by multiple devices will successfully reach the receiver with minimum interference [67].

A11: We developed an analytical model for the reliability in multi-hop molecular nanonetworks that utilise virus particles as an information carrier. This allows us to assess the effects of diffusion, absorption and decay on reliability and transmission time for multi-hop transmission.

A12: We analysed the performance of single path, defined multi-path and random multi-path topologies in virus-based nanonetworks. We compare each topology in relation to transmission delay, reliability and transmission time variance [65].

Chapter 2

Related Work

This chapter presents related and published work in the domain of this thesis. The work is divided into two subsections. Section 2.1 presents related work in molecular communication. Section 2.2 describes the related work in synthetic biological components. The works described in this section combine both biological and networking concepts. A good introduction to biological concepts for computer scientists can be found in [110]. Similarly, a good description of general networking concepts can be found in [109].

2.1 Molecular Communication

In Section 2.1.1 and 2.1.2 we present state-of-the-art solutions of wireless and wireline paradigms in molecular communication. In section 2.1.3 we review work related to the characterisation of molecular communication channels from an information theory perspective. Finally, in section 2.1.4, we review work that contributes to the development of nanonetwork protocols.

2.1.1 Wireline

We classify wireline molecular communication as solutions that use a separate physical mechanism to guide the transportation of message molecules (an analogy being copper wire or optical fibre interconnections that guide electrons or light respectively). Several mechanisms exist in nature that provide natural, wired transport directionality for molecule payloads that can be re-engineered to create nanonetworks. An example of this is provided by Onfelt et al. [80] who induced a small network of membrane nanotubes which were used to transport molecules between interconnected cells. Similar to this approach, in [32] Moritani et al. proposed the transport of message carrying vesicles between nanomachines on a micro lithographic track containing motor proteins. In [79] Enomoto et al. also use microtubules to connect between different nanomachines, similar to the wireline interconnections found in conventional communication networks. In Enomoto's solution, the transportation process is conducted through molecular motors that walk on the microtubules. Cytoskeletal filaments are used inside cells to transport molecules and have been utilised to create an architecture of rail molecules upon which molecular communication can occur [78].

These wireline approaches provide a direct physical connection between sender and receiver and can operate in a unicast mode. These solutions are not affected by dilution, distance or noise in the same way as wireless solutions and they tend to be more energy efficient in terms of transmission (i.e. they do not need to transmit large quantities of message molecules). On the other hand, there is a requirement to construct the nanonetwork infrastructure of rail molecules, after which the topology will be fixed. This may not suit some applications that would require a more flexible network that can support changes such as mobile nodes. In the next section we discuss the wireless approach which provides these features.

2.1.2 Wireless

Conventional wireless devices communicate typically using radio waves that propagate through space. From a molecular communication perspective, a number of approaches have been investigated that mimic the propagation of radio waves using molecules. The transport of signal molecules in wireless molecular communication can be classified as passive or active. In passive communication, the signal molecules diffuse via Brownian motion in the communication channel [76]. Active transport involves some form of propulsion using chemical energy and propagation in a particular direction, ideally towards the intended receiver [74]. The following sections discuss the state of the art in both approaches.

Passive Transport

Much of the research in wireless molecular communication is based on the molecular diffusion process whereby a transmitting node emits a quantity of molecules which diffuse passively to the receiver node. Consequently many analytical models for diffusion-based molecular communication use solutions to Fick's first and second laws [34]. In the simplest form of passive transport, a bionanomachine releases signal molecules into the communication channel and they diffuse in all directions and may come in contact with the receiver. For example, vesicles containing message molecules can be released from engineered cells and deliver their payload to other engineered cells with complementary cytoplasmic receptors [33][88].

In channels consisting of tightly packed cells, signal molecules can also move from cell to cell through gap junction channels [77][104]. Gap junctions are physical channels that can connect adjacent cells and can control the movement of molecules between the cytoplasm of each cell. This type of communication can also propagate using a "fire-diffuse-fire" mechanism which is typically found in calcium ion-based solutions. Diffusing molecules can cause a sudden release of molecules in each cell, giving the impression of a wave of calcium ions propagating through the channel. For example, in [13] Nakano and Liu investigated calcium signalling for diffusion-based molecular communication in a relay channel. The authors provide analysis from an information theory perspective including the calculation of information transfer rate, and its dependence on the emitted molecule concentration, as well as distance of propagation. Simulation results are based on a one dimensional lattice of cells connecting a receiver and transmitter. A minimal intracellular calcium model is combined with the Monte Carlo-based Gillespie simulation method to achieve a compartment-based

simulation of diffusion. In [16] Guney et al. present the concept of a mobile ad-hoc molecular nanonetwork (MAMNET). This concept is based on mobile nanomachines sharing information at the nanoscale through intermittent physical contact as they diffuse in an aqueous environment. The analytical framework focuses on the effects of Brownian mobility on the performance of electrochemical communication among nanomachines. Numerical analysis of the model suggests adequate high throughput with acceptable communication latency. Much of the simulation work in passive channels is in one dimension. This somewhat restricts its application for simulating more complex diffusion-based nanonetworks in two or three dimensions. One possible approach to extend the model in [13] to more realistic and complex multi-dimensional environments is to use the Gibson-Bruck algorithm [14], an extension of the Gillespie algorithm for simulating more complex biochemical systems. Furthermore, MonteCarlo simulation is often restricted to simpler simulation scenarios due to computational demands. The analytical model proposed in [13] could be extended to more complex random networks of multiple receivers and transmitters by using the computationally inexpensive Fire-Diffuse-Fire model for calcium signalling [15] which also uses the compartmentalised simulation approach.

Active Transport

Chemotaxis describes the ability of bacteria to direct their movements in reaction to particular sensed chemicals in their environment [83]. This characteristic has enabled the development of molecular communication solutions which use bacteria [33][81][82]. For example, in [17] Lio' and Balasubramaniam propose a multi-hop routing mechanism which utilises bacteria as message molecule carriers for a network of nanomachines. Lio' et al. base their solution on specific biological processes, modelling the natural tumbling and chemotaxis behaviours of bacteria, to provide active transport for message molecules (in this case DNA) in the direction of the intended receiver. They also use the conjugation property of bacteria to mimic the mechanism of Delay Tolerant Networks (DTN) found in mobile networks. In [69], this concept is further developed to create a multi-hop molecular communication nanonetwork where opportunistic encounters are used to pass messages via the conjugation process.

2.1.3 Channel Characterisation

The characterisation of molecular communication channels is a key research area in molecular communication nanonetworks [11][62] and there is an increasing volume of research, both from a machine capacity and channel capacity perspective [70][72][73][74][75][103], much of which is based on Shannon theory [84]. For example, in [85] Schneider characterises the machine capacity of molecular scale machines. In doing so, Schneider is able to calculate the information capacity that must be approached by the communication channel. The author's work, based on mRNA molecule processing, proposes that Shannon theory can be applied to nanomachines and, consequently, that reliable communication between molecular machines (in our case bionanomachines) in the presence of thermal noise can be achieved once sufficiently complex encoding algorithms can be implemented at this scale.

In [8] and [86], Pierobon and Akyildiz investigate the achievable end-to-end

capacity using molecular diffusion. This included a general mathematical model for diffusion-based molecular communication which was used to analyse the impact of noise on channel capacity. This work is extended in [87], where a mathematical model for noise associated with the ligand-based reception of molecular communication reception signals is presented. This analysis concentrates on a general model for diffusion-based molecular communication and does not focus on any particular molecular communication model. However, in [13] Nakano and Liu develop a channel communication model for calcium ion signalling from an information theory perspective. Using accepted system biology models for calcium mechanisms, the authors are able to calculate the information transfer rate and show its dependence on the concentration, as well as distance, between sender and receiver.

The physical link distance is a common constraint for reliable communication in biological nanonetwork channels. This distance could be important for routing decisions in multi-path multi-node nanonetworks, just as it is used in some sensor network protocols [18]. In [19] and [107], Moore et al. propose techniques for bionanomachines to measure the distance to other bionanomachine which can be used to inform communications-based functions in nanonetworks. Several protocols for distance measurement are proposed, analytically modelled and compared. However, several assumptions are made that will need to be addressed in future work. First, it is assumed that the receiver can measure accurately the time and concentration of returned signal. This will require the development of suitable protocol components that can interface with, and accurately sense, calcium ion concentrations. Secondly, the simulation scenario is between one transmitter and receiver in a one dimensional space. Again, for more complex nanonetworks or MAMNETs, several nanomachines may be within an effective communication distance which could result in signal interference and inaccurate distance measurement (similar to that shown in our research). Thirdly, in single spike feedback, the distance measurement protocols are based on concentration measurement. For multiple access, multi-node nanonetworks, this protocol will also need an addressing mechanism to identify the bionanomachines in range. Moreover, general addressing mechanisms are required for biological nanoscale networks.

2.1.4 Biological Protocol Functions in Nanonetworks

In [39] Sauro et al. propose that, similar to microelectronics fabrication, biological networks have also evolved modularised functions and mechanisms to address complex signalling systems. In this section, we examine the work that uses biological functions in a modular way to create the components for molecular communication nanonetworks. Much of the research in nanonetworks is concerned with physical layer characteristics, dealing with channel capacity, throughput and delay. However, some research has been devoted to upper data link and network layer function.

Biological Interfaces

The concept of an interface definition is a key characteristic of networked distributed systems. Interfaces provide an abstraction that can be re-used by several communicating processes. One approach to this is the use of an encap-

sulating biological mechanism that allows the transport of arbitrary molecule payloads. For example, in [88], Moritani et al. use vesicles to encapsulate and transport molecular payloads between bionanomachines. A similar approach, although not developed specifically for nanonetworks, is the use of viral vectors for drug delivery in biomedical therapies [6] that separate payload (drug) from the transport mechanism (virus particle). Transport vectors act as an abstract nanoscale communication interface and do not use the characteristics of the payload (i.e., the information molecule) being transported. Furthermore, they can provide an addressing function through their surface ligands which will be discussed in the next section.

There is also a requirement to connect the functional components of molecular communication systems, such as to interconnect a sensor application layer function to a communication/network layer in order to communicate the occurrence of a significant event. One approach is to use aptamers which are DNA or RNA sequences that are attracted to and bind to specific target molecules. In [101], the authors use aptamers to create biochemical-based logic circuits with the intention of controlling the delivery of drugs in future biomedical therapies. In [100], An et al. re-engineer the shRNA pathway to create a cell-based molecule sensor by including an aptamer region in the shRNA. The aptamer will bind to any target molecules present in the cell and can then, in turn, modulate the operation of other functions in a cell, for example the encoding of message molecules. Thus aptamers can be used to specify external interfaces for nanonetwork components in a cell. Furthermore, it is possible to produce separate shRNA pathways where the aptamer of each of the shRNAs can target two different molecules. For example, nanonetwork components could be engineered to detect and respond in the same way to two different molecular inputs, providing the abstraction and reuse characteristics used in traditional component-based approach in distributed systems.

Addressing in Molecular Communication

A key property of molecular communication nanonetworks is an addressing mechanism to provide the ability to send information to a specific bionanomachine connected to the network. For wireless concentration-based nanonetworks, addressing can be based on the molecule species or the specific molecule conformation being used [20]. However, this approach would require a specific molecule or molecule conformation for each address in a multiple access network. Furthermore, the transmitting nanomachine must have the capability to produce the distinct molecules for each address. Another approach is to use molecular concentration as an addressing mechanism. For example, in [21], Mahfuz et al. suggest concentration dependent addressing for diffusion-based molecular communication, i.e. a transmitter can selectively choose which of the receiving nodes it would like to communicate with by varying the number of transmitted molecules. This is similar to our solution in [60] which also includes simulation results using calcium models and takes into account the simultaneous transmission in multiple access networks. However, the scalability (number of distinct addresses) of this approach will be limited both by the maximum range concentrations that can be produced at the transmitter and by the fidelity of the concentration at the receiver.

A more generic approach is to have a separate and generic addressing mech-

anism in the communication process similar to encapsulation in conventional communication protocol stacks. This is more scalable and allows transportation of different payloads to specific locations in the network. Biomedical viral vectors already provide a generic interface as discussed in the previous sections and can also use specific ligands on their surface that attach to receptors on their targets. The increasing sophistication of this type of genetic engineering continues and it is now possible to engineer cells that can encapsulate several distinct molecular payloads into a vector [22]. Currently, as far as we are aware, the use of these therapies are not fully autonomic *in-vivo* and involves a manual titre of the vectors before their introduction into their target containing environments.

Due to their ability to form bonds through hybridisation, nucleic acid molecules also provide a means of biological addressing. For example in [23] and [47], Hiyama et al. use single-stranded DNAs (ssDNA) attached to message molecules. The ssDNA specifies the receiver address and the receiver bionanomachine exposes a complimentary DNA molecule designed to attach to the respective single-stranded DNA on the information molecule. A key benefit of using nucleic acid molecules for addressing is the massive scalability of possible addresses, which will facilitate the creation of more complex molecular communication networks. Furthermore, the binding of DNA sequences is well understood from research into self forming DNA nanostructures [24].

The next section reviews mechanisms and approaches that can be used to create molecular communication protocols using synthetic biological components.

2.2 Synthetic Biological Components

In this section we review the state-of-the-art in the creation of biological components that could support the implementation of protocols for molecular communication nanonetworks. Much of the work described has inspired the solutions and achievements in the thesis and can be classified under the broader domain of *Synthetic Biology*. Synthetic biology is the design and construction of biological components such as genetic and enzyme-based circuits, or the re-purposing of existing biological systems or cells [54][42][49]. A distinct characteristic of synthetic biology from traditional molecular, cellular and systems biology is the focus on the design and construction of core components. The following sections review this area from the point of view of nanoscale computation.

2.2.1 Nucleic Acid-Based Computation

Nucleic acids are seen as biology's information molecule and have been used to create information storage solutions through synthesised DNA molecules. For example, in [25] the authors developed a rewritable addressable data module that switches between states in response to distinct inputs, thus demonstrating data storage in DNA. Recently, DNA was used to encode and retrieve computer files totalling 739 kilobytes with potential for greater volumes [102]. Nucleic acid molecules are a key facet in molecular communication nanonetworks and their potential goes beyond information storage to creating interfaces between distinct chemical processes and computation. For example, artificially engineered nucleic molecules are used to create and self-assemble complex nanoscale structures and

machines [26][99]. Furthermore, simple algorithms such as an adder and subtractor [27] have been implemented using nucleic acids, utilising primarily the predictability and affinity of how nucleic acids bind to each other. For the most part, these applications operate in vitro and separate from the cell-based genetic mechanisms. However, the creation of synthetic biology systems *in-vivo* using existing biological mechanisms and genetic pathways provides the advantage of interfacing directly with other biological systems. For example, in [30], Wiess et al. construct computational components using cell-based DNA transcription and translation processes. The authors engineer a biochemical inverter using input mRNA which generates a repressor protein that prevents DNA transcription processes. In the absence of the input mRNA, DNA transcription processes proceed to generate an output mRNA. In [28] and [31], Xie et al. show how *in vivo* "molecular computers" could reuse biological processes and create a programmable unit which implement biological computation. These solutions modify cellular RNA pathways to engineer logic circuits. Moreover, these approaches show increasingly complex circuits that can be used as a platform for *in-vivo* molecular computing. Similar to the approach in [30], in [29] Rinaudo et al. use RNA interference (RNAi) pathway and short interfering RNAs (siRNAs) to block/allow specific gene expression in response to a set of molecular inputs. In doing so, the authors implement synthetic circuits in the cell. This provides a more generalised technique of creating logic circuits that can be integrated to specific gene expression. What is particularly advantageous in this approach is the generic ability to adapt these solutions to different inputs. In this research, we use this modularity as a mechanism to interface protocol layer components to upper layer application sensor mechanisms and to molecular communication functions at the lower layers.

2.2.2 Enzyme-Based Computation

Cell-based biochemical computation can also be approached through the use of enzyme pathways and reactions. For example, Mitogen Activated Protein Kinase (MAPK) [40] is an important cellular signalling mechanism which is highly conserved in almost all eukaryotic cells. MAPK has been extensively modelled analytically [35][36] and is of particular interest in biochemical computation due to its ultrasensitive behaviour: MAPK pathways are less sensitive to small stimuli but exhibit an abrupt, switch-like response to stimuli above a certain threshold. This property is used by Markevich et al. in [37] to create a bistable switch using a MAPK pathway. Similarly, in [38] Stetter et al. utilise the bistable nature of biochemical enzymatic reactions to create a reusable, "easy to engineer" architecture that forms the basis of several Boolean logic functions such as AND, and OR gates. However, creating complex in-vivo circuits has challenges such as chemical heterogeneity, uniformity and predictability [39]. Niazov et al. [43] successfully orchestrated a series of interconnected logic gates based on similar enzyme reactions. To achieve modularisation, each logic sub-unit must employ compartmentalising mechanisms, for example a distinct chemical species set to prevent intrinsic chemical interference between gates. This approach is also taken in [44]. One constraint of this approach is the requirement to find an adequate number of enzymes to create more complex circuits and this is the subject of future work by these researchers. Another approach to providing complex biocircuits is scaffolding molecules [41][68] which can be used to

compartmentalise enzyme reactions. These mechanisms can provide computational functions to support nanoscale computation for molecular communication nanonetworks.

2.3 Discussion

The multidisciplinary nature of this research domain is resulting in a convergence of research approaches from separate domains. The experimental nature of biology research is now being influenced and informed by typical approaches in computer science and technology, particularly in the area of modelling and simulation. For example, the emergence of SBML, an XML-based modelling language and the component approach in synthetic biology are good examples of mature computer science techniques being adopted in biology research. This convergence also facilitates the emerging paradigm of nanonetworks. Significant and mature research exists in the synthetic and systems biology domain. This provides an excellent opportunity to develop protocol designs for create molecular communication protocols. For example, this thesis uses existing models from system biology to develop MAPK pathway-based enzyme logic circuits to control calcium signalling molecular communication. We characterise the pathway performance for use as logic circuits. We also use SBML representations of intracellular calcium and develop a SBML model for RNAi interference-based circuit to control virus particle production in a biological cell. These models are used to simulate protocol components of molecular communication nanonetworks. It is clear that these approaches, combined with existing advances in channel and nanonetwork characteristics, will provide an important platform for research in the ongoing evolution in nanonetworks.

The previous sections describe the current landscape of molecular communication research and demonstrate significant and exciting results, both experimentally though lab experiments and theoretically though the development of mathematical information theory models. This work provides the basis for the creation of a physical layer for molecular communication nanonetworks. Similarly, progress in synthetic and systems biology is providing the computational ability to control communication between interconnected bionanomachines. Furthermore, the open access approach to research in the synthetic biology community (known as Science 2.0 [89]) is beginning to gain popularity. For example, the Biobricks Foundation [90] and the Biomodels Database [91] are providing an open collaborative approach to biological development in a way that is similar to the open source movement in software development [55]. What is interesting is the pattern emerging across these multidisciplinary research domains. The fusion of telecommunications and computing technology formed a basis for modern data networks, the Internet and the explosion of applications that followed. We believe the fusion of molecular communication and synthetic biology will create the foundation upon which molecular communication nanonetworks will realise their full potential and enable future applications. A key aspect of this is communication protocols.

To our knowledge, there have been no solutions for communication protocols specific to molecular communication nanonetworks. The works contained in section 2.1 concentrate primarily on the vital task of channel characterisation and exploring experimentally the possible mechanisms to conduct molecular

communication. This work is now reaching a level of maturity where the next logical step is to develop suitable communication protocols for bionanomachines. Here we can take advantage of the works and tools emanating from the systems biology and the synthetic biology domain to explore communication protocols *in silico*.

Chapter 3

Research Summary

This chapter summarises the findings of the research presented in this thesis, where we present our conclusions (Section 3.1) and then provide some topics that could serve as a continuation of the research (Section 3.2).

3.1 Conclusions

The conclusions are presented as answers to the research questions in section 1.2.2 and are as follows:

3.1.1 RQ1.*: Mapping conventional protocols to molecular communication protocols

RQ1.1

Communication protocols have tremendous potential for molecular communication nanonetworks. However, the direct implementation of existing communication protocols is not possible due to the physical constraints of bionanomachines, biological components and the challenges inherent in the biological medium. This thesis addresses this by applying synthetic biology solutions to develop and evaluate new protocols for molecular communication nanonetworks. In [59] we proposed a bi-layered protocol stack consisting of a limited encoding and error recovery function, based on natural compartmentalisation techniques. Furthermore, our solution includes a set of loosely coupled protocol components, instead of a generic protocol stack, to prevent unnecessary complexity in restricted bionanodevices [64]. Our simulation results highlighted the challenges in protocol implementation due to noise and chemical instability.

RQ1.2

The solutions presented in the output of this thesis demonstrate the necessity of matching the characteristics of each molecular computing technique to the computational requirements of each layer of the proposed protocol stack. In the case of nucleic acid-based protocols, we conclude that this approach is suitable to molecule encoding functions and error correction functions mainly because more complex computation is possible. Our use of nucleic acid-based automaton

illustrated this and we took advantage of the DNA restriction process to provide addressing. Furthermore, to counter-act the complexity of connection orientated communication, we also provide a FEC solution through the use of DNA affinity to correct encoding errors.

RQ1.3

A major constraint in molecular computing is the difficulty in linking molecular-based logic components to create the complex logic required in communication protocols [105]. Furthermore, as we have shown in our published output, molecular reactions can interfere with each other in a similar way to electromagnetic crosstalk in conventional networks. For enzyme-based protocols, we found that although it is possible to conceptually create complex logic, practical implementation of such circuits is difficult due to lack of linkable enzyme reactions as discussed in section 2.2. Also, the characterisation effort required to achieve enzyme computing increases dramatically relative to circuit complexity as we demonstrate in [43]. We believe this makes enzyme computing a suitable choice for relatively simpler protocol function that require short, responsive computation time.

3.1.2 RQ2.*: Utilising biological cells as a nanonetwork communication platform for protocol development

RQ2.1

Enzymatic pathways are intrinsic in cytosolic cell signaling pathways [38] and, in addition to the speed and simplicity features of enzyme computing identified in RQ1.3, the MAPK pathway has the potential to link with extracellular events via cell membrane components such as receptors and gap junctions to other cell components. In [60] we use this feature of the ultrasensitive MAPK enzyme cascade model to functionalise an application layer protocol component to control calcium signalling network layer. This makes it particularly suitable to detecting extra-cellular input events.

RQ2.2

The RNAi-based protocol provides the possibility of richer information encoding through molecular encoding of mRNA nucleotides. This approach will suit applications that require rich data encoding capabilities. Our work has also shown that this form of computation is much slower relative to cytosolic-based enzyme cascades and would be most suited to a slow transmission rate of messages that contain a high density of information. Also, in relation to using this approach to control a virus-based network layer, the time to activate a significant quantity of viral particles is long relative to other molecular communication modes (in the order of days). Furthermore, a high level of coordination is required for the many steps involved in the protocol.

RQ2.3

External interface definitions are a key characteristic of software component development. Similarly, aptamer molecules can be used to specify external

interfaces in RNAi-based logic solutions. The SELEX [106] method can be used to identify aptamers that bind to specific target molecules and thus link their occurence to RNAi logic circuits. We use this approach to introduce modularity to RNAi-based protocol functions which is a defining trait of synthetic biology.

3.1.3 RQ3.*: Analysis of scheduling and routing in molecular communication nanonetworks

The physiochemical and biological properties of the components that are used to create molecular communication nanonetworks have a major bearing on the architectures and topologies of nanonetworks. As already discussed in section 2.1.2, there is ongoing work in creating models for molecular communication channels from an information theoretic point of view. The heterogeneous nature of this environment proves difficult to model however, existing models are constantly evolving. The work in this thesis also demonstrated the effect physiochemical properties can have on network reliability and why they need to be considered in protocol development and topology design.

RQ3.1

The diversity of molecular communication modes, and their corresponding characteristics, pose a significant challenge to protocol development. This shows how we need to tailor protocol solutions for molecular communication modes. In [63] we demonstrated the effects of neuron refractory time in various topologies of neuronal networks. In [68], inspired by time division multiple access protocols in embedded systems, we demonstrated how blocking can be minimised using a genetic algorithm-based approach to message scheduling. These results point to the condensed, loose composition of protocol components proposed in other research output [60].

One good application of neuronal nanonetworks that illustrates the need for the solution in [63] could be a sensor network designed to detect the occurrence of a toxin or chemical event in a living tissue (similar to the applications proposed in [3]). If detection of a single event results in a response at the receiver then collision avoidance and blocking are not required. However, the response of the receiver may depend on the aggregation of received signals over time. For example, the frequency and quantity of signals received at the receiver can be used to actuate a graduated response. Therefore, the reliable delivery of all signals is required and could be achieved using the GA approach in [63].

RQ3.2

A key consideration in the development of molecular communication nanonetworks is the physiochemical and biological characteristics of the communication channel. Furthermore, heterogeneous nature of different channel types and deployment environments do make the development of communication networks to transport data from the source to destination challenging. In [65] we propose an analytical model to calculate the reliability and delay of multi-path virus nanonetworks using different physiochemical properties. Our numerical results indicate that particle dilution and diffusion speed of virus particles can have a significant and, in some cases, counter-intuitive effect on transmission delay

and reliability. Furthermore, the replication latency in intermediary nodes dominates the overall end-to-end transmission delay. As a result, large transmission delay times will occur on paths containing many intermediary nodes. This will, more than likely, be the case for all molecular communication protocols that use genomic-based functions to process and replicate messages.

Our analysis of virus-based communication, associated with achievements A11 and A12 in section 1.4, is in two dimensions. While this affects significantly the spatial communication range and maximum molecule concentration relative to the corresponding three dimensional model, we choose two dimensional model for our work as it is likely that comparable simulation and experimental work will take place on a two dimensional in-vitro lattice of cells. The proposed model can be converted by using the three dimensional solution to the diffusion equation in the analytical model. Furthermore, it should be noted that the reliability model developed in [65] and [66] are generic and can be applied to other diffusion based molecular communication channels other than virus-based transport. In order to do this, it would be necessary to adjust the physiochemical parameters to suit the molecular communication mode in question. For example, using calcium ions would require the use of a diffusion coefficient of $30\mu m^2 s^{-1}$, which would results in a faster average time to reach the receiver. Of course other parameters would need to adjusted such as receiver absorption rates.

RQ3.3

The contrasting characteristics of molecular communication, compared to conventional networks, means that the topology and architecture of molecular communication nanonetworks can have a significant affect on communication performance. In [63] we investigated four topologies of neuronal-based network with multiple access and the blocking rate was calculated for various numbers of bionanodevices and transmission rates. This allows us to determine how particular transmission patterns perform in various topological shapes. Similarly in [66] we evaluate single path and redundant path topologies for virus-based networks. We demonstrated equivalent reliability is possible in single path with implicit acknowledgement and defined multi-path topologies for virus nanonetworks. However, transmission delay for single path with implicit acknowledgement is significantly higher than multipath. This is also borne out in the neuronal solution where defined multi-path routes show better performance (less blocking) than single path topologies. This is somewhat obvious, yet we have proposed solutions to achieve a similar performance level in single path for both neuron and virus approaches (scheduling for neuron, implicit acknowledgement for virus).

3.2 Future Work

We would consider that the following topics are the most likely areas for the continuation of the research presented in this thesis.

Network Coding in Molecular Communication Nanonetworks

As shown in this thesis, communication in molecular communication nanonetworks is characterised by a long propagation delay and poor reliability. Our

work also showed that using conventional acknowledgement and re-transmission protocols can increase, considerably, the propagation delay and are limited by the computational constraints in bionanodevices. Network coding approaches, which are used in some wireless network applications [108], could be used to minimise transmission time and increase end-to-end reliability in nanonetworks. Furthermore, the network coding methods may be compatible with nucleic acid molecular computation approaches used in this thesis.

Tissue Area Nanonetworks

Communication networks are continually evolving and becoming ever more pervasive with solutions such as LAN, WLAN, body area networks (BAN) that use standards such as Bluetooth, IEEE 802.11, Zigbee etc. The use of the communication protocols and networks described in this thesis will facilitate the creation of Tissue Area Nanonetworks (TAN): biological nanoscale communication networks that could be integrated in living tissue. A TAN would consist of an interconnected network of bionanomachines acting as sensors and actuators. The sensors and acuators could be engineered cells, using suitable communication protocols, to perform healthcare applications inside the human body. Moreover, the ability to interconnect TAN networks with existing communication networks would allow healthcare professionals to monitor and control the bionanomachine activity via the internet. This would require the development of a suitable gateway with a micro-interface (micro-gateway) to the TAN networks. The creation of these gateways would facilitate the concept of the "Internet of NanoThings".

Internet of Nano Things (IoNT)

The Internet of Nano Things (IoNT) will see the advent of novel software services that would be distributed and embedded in micro-gateways that interface to the nanonetworks and collect information. These services will receive and analyse data from a large number of bionanosensors and will require the ability to deal with the large diversity of data semantics that exist in molecular communication nanonetworks (e.g. concentration encoding of specific molecule species compared to molecular encoding in nucleic acids). Dealing with this diversity is hugely challenging and provides an avenue for the extension of the work in this thesis. The realisation of the IoNT will also expose the currently isolated domain of BANs and nanonetworks to the inherent security threats present in the internet.

Security

Security is a key issue for molecular communication nanonetworks. Applications using molecular communication nanonetworks will be susceptible to corruption and attack similar to conventional networked applications. In [64] we refer to DNA strands being used as the "software" for a biological communication protocol. Therefore it is conceivable that malicious DNA "software" could be targeted at bionanomachines, via nanonetworks, to disrupt their normal operation (ironically using viral vectors as a possible delivery vehicle). This could have serious consequences particularly in the context of the biomedical applications discussed in previous sections. Furthermore, the implementation of IoNT

using molecular communication nanoneworks in the body not only exposes the nanonetwork and connected bionanomachines, but potentially exposes other unrelated biological processes and networks that exist in the body. However, the opportunity now exists to incorporate security at an early stage of the domain's evolution. Therefore secure and robust nanonetworks should be at the forefront of current nanonetwork research challenges. One obvious approach is to investigate the mapping of existing security paradigms, particularly those used in wireless sensor networks, to nanonetworks. Possible areas for investigation are: intrusion detection, integrity protection and encryption mechanisms. Also, just as molecular communication is inspired by biological communication, novel and compatible security solutions for molecular communication nanoneworks may emanate from naturally evolved biological defence and immune systems. For example, scaffolding of enzyme pathways proposed in [59] could be explored as a means to protect the protocol logic from malicious manipulation.

Molecular communication nanonetworking is a novel approach to communication networks that mandates a truly interdisciplinary approach. We believe that, in addition to contributions from the domain of biological science, the domains of computer science and engineering will be a key contributor in the evolution of molecular communication nanonetworks.

Chapter 4

Publications

This chapter presents the complete list of published research articles of the author which are relevant to this thesis. The articles are listed in chronological order and their full text is available in the appendix.

Published Articles

P1 F. Walsh, S. Balasubramaniam, D. Botvich, W. Donnelly and S. Sergeyev, "Development of molecular based communication protocols for nanomachines", Proceedings of the 2nd international conference on Nano-Networks. ICST (Institute for Computer Sciences, Social-Informatics and Telecommunications Engineering), 2007.

P2 F. Walsh, S. Balasubramaniam, D. Botvich, T. Nakano and T. Suda, "Simulation framework for communication protocols of molecular communication systems", Proceedings of the 3rd International Conference on Bio-Inspired Models of Network, Information and Computing Sytems. ICST (Institute for Computer Sciences, Social-Informatics and Telecommunications Engineering), 2008.

P3 F. Walsh, S. Balasubramaniam, D. Botvich, T. Suda, T. Nakano, S. F. Bush, and M. Ó. Foghlú, "Hybrid DNA and enzyme based computing for address encoding, link switching and error correction in molecular communication", Proceedings of the 2nd international conference on Nano-Networks", ICST (Institute for Computer Sciences, Social-Informatics and Telecommunications Engineering), 2009.

P4 F. Walsh, S. Balasubramaniam, D. Botvich, and W. Donnelly, "Synthetic protocols for nano sensor transmitting platforms using enzyme and DNA based computing", Nano Communication Networks, vol. 1, no. 1, pp.50-62, 2010.

P5 F. Walsh, N. T. Boyle, A. Della-Chiesa, A. Mardinoglu, D. Botvich, A. Prina-Mello and S. Balasubramaniam, "Artificial backbone neuronal network for nano scale sensors", Computer Communications Workshops (INFOCOM WKSHPS), IEEE Conference on, 2011.

P6 S. Balasubramaniam, N. T. Boyle, A. Della-Chiesa, F. Walsh, A. Mardinoglu, D. Botvich, and A. Prina-Mello "Development of Artificial Neuronal Networks for Molecular Communication Nano Communication Networks", Nano Communication Networks, vol. 2, no. 2-3, 2011.

Articles accepted for publication

P7 F. Walsh, S. Balasubramaniam, "Reliability of multi-path virus nanonetworks", Computer Communications Workshops (INFOCOM WKSHPS), IEEE Conference on, 2013. (ACCEPTED)

P8 F. Walsh, S. Balasubramaniam, "Reliability and delay analysis for multi-path Virus-based Nanonetworks", IEEE Transactions on Nanotechnology. (ACCEPTED)

Appendix A

A.1 Development of molecular based communication protocols for nanomachines

This initial publication focuses specifically on protocols for molecular communication. We concentrate on identifying published synthetic biology solutions that can be used to implement the protocol logic required in a general molecular communication channel. We used this to design an explicit acknowledgement protocol for reliable communication between nanomachines. Furthermore, through simulation, we highlight the challenges in creating complex logic circuits for protocols using enzyme-based computation.

Development of Molecular based Communication Protocols for Nanomachines

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ABSTRACT

Communication between nanomachines (e.g. biological nano-scale devices) has the potential to open up new opportunities and applications, especially in areas such as health care and information processing. Inspired by current data communication protocols, we propose a molecular-based communication protocol to enable reliable communication between nanomachines. In this paper, we present a protocol stack which enables nanomachine communication through the encoding and transmission of data as biomolecules. Extending previous work in logical nano-computation, we define an error-recovery process through the use of a chemical state machine based on nano-logic circuit. Finally, we present initial results of simulation of nanomachine communication in the presence of both channel and chemical instability and demonstrate the performance of the nano-circuit in such conditions.

Keywords

Nanomachine, Nano-Communication, Communication Protocols, Molecular Communication, Nano-Computation

1. INTRODUCTION

The increased focus on nanotechnology in recent years has accelerated medical research towards the use of nano-devices in medical applications. These medical applications include the use of nano-scale devices to develop new drug delivery methods, cancer detection, diagnosis and treatment, assembly of macromolecules, and monitoring and supporting regulation within the organism. The focus of our research is on the development of communication protocols for nano-devices based on reusing protocols from telecommunications and data networks.

Nanodevices are small scale devices that are manipulated from materials of one billionth of a meter size [7]. Nanotechnology has progressed beyond plain particles with embedded chemicals to where new nanomachines are emerging with nano-computing capabilities. A good example is nanorobots, where [1] developed the concept of nanorobots to assemble macromolecules. However, a key requirement in ensuring the efficient functionality of nanomachines is the cooperative capabilities between the various devices, due to their sheer size and processing power. To support this capability a key requirement is communication mechanisms between the various disparate nanodevices. Since nanodevices have limited processing capability, functionalities must be performed in cooperation with their environment. In this paper we propose a communication protocol for nanomachines in a biological medium. Our solution is based on a protocol stack which allows the nanomachines to encode the data bits into biomolecules, and support transport protocol such as transmission and error recovery through the control of nano-logic computation manipulated within biological cells. In this paper we focus particularly on investigating the use of transmission and error recovery protocol from data communications using molecular nano-logic computing. An example application of our proposed communication protocols is for communication amongst nanorobots [1]. This capability can be achieved by enabling nanorobots to migrate and attach themselves to cells and control the logic operations for communications. The robots can then migrate and interface to a different cell and perform the same communication operation.

The paper is organised as follows: Section 2 presents the related work on molecular computing and communication. Section 3 presents our overall solution, while section 4 presents some simulation results to illustrate our idea. Lastly section 5 presents the conclusion.

2. RELATED WORK

2.1 Molecular Computing

Stetter et al [4] developed a mechanism to allow logical nano computation to be performed by manipulating the concentration of enzyme molecules. Manipulation of enzymes includes mechanism of kinase activation to allow an enzyme to be active, while de-activation is performed through phosphorylation of the enzymes. This leads the enzymes to exhibit bistability behavior. From this behaviour, a number of solutions were proposed by the authors (e.g. fan architecture, linear cascade) which allowed for logical computation to be performed as AND, OR, and NOT gates.

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2.2 Molecular Communications

Enomoto et al [2] have proposed a molecular communication solution for nano-scale communication for nano-devices. The molecular communication allows devices to encode and decode information into molecules and to transport this information to peer nano-devices. The solution is inspired by biological systems that communicate using molecules, where molecular communication using molecular motors was observed within a biological cell. The authors seek to develop an engineered molecular communication system employing various processes (encoding, sending, propagating, receiving, and decoding) and components (sender and receiver). The solution proposed by the authors uses molecular motors (e.g. kinesin, dynein), which are used to transport materials in eukaryotic cells along filaments referred to as rail molecules (e.g. microtubles), for controlled nanomachine communication.

Nakano et al [3] proposed a mechanism to allow intercellular communication between distant nanodevices using induced calcium signalling. The solution proposed by the authors exploits the current calcium signalling between cells, by modifying the frequency and amplitude of the calcium concentration. Through this mechanism, calcium wave propagation occurs which allows controlled intercellular communication. By interfacing a nanodevice to a specific cell, calcium communication can be formed by controlling the calcium production in the event the device has access to the cytosol of the cell. In the event that cell has no access to the cytosol, then the nanodevice can emit substances that can bind to specific receptor of the cell that can induce calcium production. The challenges relating to describing accurately a communication channel for the transmission of messages in 'wet' techniques are highlighted by Alfano et al [5] who state the 'most urgent question' for molecular communications is the characterization of the 'wet' communication channel. The authors point to the challenge posed by non-linear nature of the channel and the necessity for experimentation and computer simulation to address this.

3. PROPOSED SOLUTION

Our proposed solution is based on applying current data communication protocols and mapping these to molecular and chemical computation in biological medium. Fig. 1 illustrates our mapping mechanism.

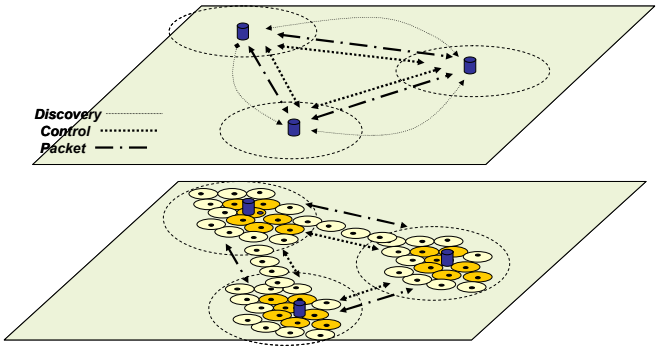

Figure 1. Overlay mapping of data networking and nano-machine communication in biological medium

As shown in Fig.1, plane A demonstrates how peer devices perform communication, where devices initially discover each other through messaging advertisement. Once devices are discovered (using ad hoc networking techniques), communication channels are established between the different devices using communication protocols to define how packet transmission can be established. Our intention is to map this process to biological medium in plane B, as shown in Fig. 1.

Our proposed solution is shown in Fig. 2, which is based on a bi-layered protocol stack. Our application is based on the ability of nanomachines to bind to a cell and trigger logical functions to perform communication.

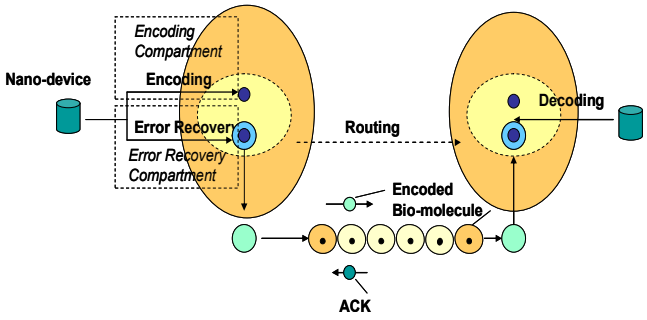

Figure 2. Communication protocol for nano-machines

The mappings to data communication are as follows: Application layer – Encoding and Transport layer– Transmission and Error-Recovery. The proposed solution aims to perform each of these processes through interconnected compartments (Encoding compartments and Transmission and Error – Recovery compartments). Each of these compartments will hold nano-logical gates that perform the specific functionalities. Within these compartments are sub-compartments that hold specific nano-gates, where the interconnection of these sub-compartments will produce a logic circuit. The subsections below will describe the processes of each compartment.

3.1 Encoding

A crucial requirement is the ability to encode the data into biomolecules and to manipulate the biomolecules when the data changes. Our mechanism of encoding information molecules is based on the concept of biomolecule computing, where certain biomolecular machines within the cell can act as automaton on encoded information [6]. A function of an automaton may include the ability to scan through a tape of symbols encoded in a biopolymer such as DNA strand, and modify certain symbols within the tape. An example of this process is illustrated in Fig. 3. The example of Fig. 3a shows a biomolecule that is segmented into an address string as well as a functionality string. By using state tables, shown in Fig. 3b, the manipulation of the string can be performed through rotation of state tables. Benenson and Shapiro [6] described the use of DNA and RNA polymerase, ribosomes, and recombinases that could be used to perform automaton functionalities. Through the defined state transition tables, the automaton process can scan specific cells of the tape and manipulate and change the encoded information (e.g. Fig. 3a shows how to change from address 1 to 2, where cell 1 is changed by using the rules of the state transition and rotating through the address state table – Fig. 3b). This encoding process will be performed in the encoding compartment before transmitting to the Transmission and Error recovery compartment for transmission.

3.2 Transmission and Error Recovery

Similar to the encoding process, the transmission error-recovery process is also based on using molecular computing techniques. Our solution is based on the solution proposed by Stetter et al [4]. The solution is based on defining a finite state machine that defines transmission and error-recovery at the transport layer (since we only have two layers, the transmission layer is also in charge of propagating the molecule at the physical layer where solutions of Enomoto et al [2] or Nakano et al [3] can be used.

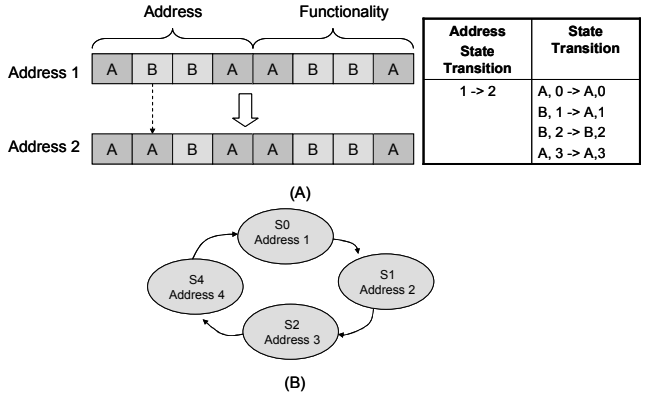

Figure 3. Nanomachine address and functionality encoding

The idea is based on defining the state machine and mapping this to a logic gate circuit. Once this is completed, a logic nano circuit is formed based on the control of various chemical concentrations based on Stetter's solution. For simplicity, we defined a simple transmission and error-recovery state machine illustrated in Fig. 4.

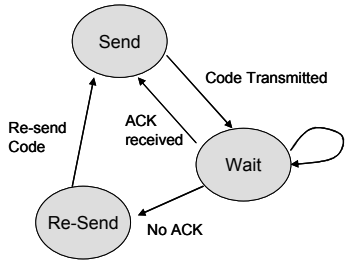

Figure 4. Error Recovery state diagram

Using the recurrent architecture for logical operations described by Stetter, the chemical state machine shown in fig. 4 can be modeled. In total, three different devices are modeled and simulated (And/Or/Not) and interfaced to create a logical circuit representing the state machine. The state machine modeled is a synchronous sequential system with behaviour that is dependant on the input and current state. Provision of a clock or cycle generation is provided by an independent chemical or enzymatic oscillation such as an autonomous calcium oscillation that resides in each molecular computer. The logic units are spatially sub-compartmentalized with output species concentrations flowing to the input of the next gate through fixed channels. Conceptually, this could be thought of as a grid of gates interconnected by channels allowing the migration of output/input chemical concentrations.

Stetter discussed the implementation of logical units based on bio-molecules using a small recurrent architecture. The basis for these logic units is the abstraction of biochemical operations catalysed by Kinases and Phosphatases [4]. In this paper we combine this abstraction of logical units with the state machine model in Fig.4 to construct a molecular computer that controls transport of messages between nanodevices. The basis for these logic units, which is devised by Stetter, is described by the following system of equations [4]:

$$\frac{dZ_1}{dt} = \alpha_{1,1} Z_1 \widetilde{Z}_1 + \alpha_{1,0} Z_0 \widetilde{Z}_1 - \beta_{1,2} Z_2 Z_1, (1)$$

$$\frac{dZ_2}{dt} = \alpha_{2,3} C_3 \widetilde{Z}_2 - \beta_{2,1} Z_2 Z_1, (2)$$

For this molecular state machine, the input concentration, for each logic unit comprises of two inputs, Z_{01} and Z_{02}. Therefore equation (1) can be rewritten in the form [4],

$$\frac{dZ_1}{dt} = \alpha_{1,1} Z_1 \widetilde{Z}_1 + (\alpha_{1,0_1} Z_{0_1} + \alpha_{1,0_2} Z_{0_2}) \widetilde{Z}_1 - \beta_{1,2} Z_2 Z_1, (3)$$

The state machine provides for connection orientated communication between two nanodevices. This communication process involves the transmission of a stack of encoded biomolecules from one nano-device to the other analogous to the 'first in first out' (FIFO) stack memory used in conventional communication systems. As we are not concerned with windowing at this stage, transmission takes place one message at a time with no requirement for packet sequence numbers. Transmission of following message biomolecules does not take place until an acknowledgement molecule is detected indicating successful delivery of the message biomolecule. Thus, we can initially avoid the complexity of congestion control, window size, and window scaling inherent in more sophisticated solutions while ensuring delivery. Admittedly this takes place at the expense of efficiency. Therefore, if an acknowledgement is not detected within a specific wait period, the last message is re-sent and a recommencement of the wait for acknowledgement is initiated.

The wait period is controlled chemically by the gradual increase in the concentration of a chemical initiated on transition to the Wait state. Once the concentration exceeds a threshold concentration the delay expiry is chemically signalled and transition to the resend state occurs. This mechanism could be constructed using the chemical bistability properties used by the logic units. Routing is reliant on the encoding of the relevant source and destination nano-machine address in the transmitting bio-molecule or packet and will be the subject of future work.

4. SIMULATION

To illustrate our proposed solution for transmission and error recovery, we have performed some simulation for biomolecule transmission. The purpose of the simulation is to illustrate mechanism of nano-machine control of molecular machines to transmit and re-transmit biomolecules in the event of message lost. Unlike conventional data networks, nano communication is a more challenging task due to the effect of errors caused by both

chemical disruption in parallel with transmission errors. This will be illustrated through the simulation.

We assume that the information has been encoded into biomolecules from the Encoding compartment and transmitted to the Transmission and Error Recovery compartment for transmission. Our mechanism for transmission is based on the finite state machine model in Fig. 4, which is created comprising of the three states and four transitions. Conventional analysis using truth tables and Karnaugh maps results in the logic circuit shown in Fig 5. Input states require two bits, S_0 and S_1 and four transition events also require 2 bits, I_0 and I_1. Each logic gate, which is a sub-compartment of the Transmission and Error Recovery compartment, is simulated using the recurrent architecture shown in Fig.5 (b) as specified by Stetter. For present purposes we will assume communication is across a channel where signal delivery success rate can be adjusted to verify the performance of the chemical state machine and simulate a noisy or lossy channel.

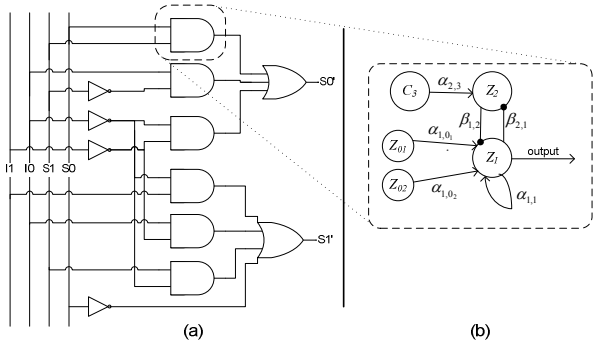

Figure 5. (a) Logic gate diagram of chemical state machine for transmission (b) Stetter's recurrent architecture that is the basis for logic gates.

In this simulation, initialization and input to the machine is performed by the associated nano-device after which it will attempt to successfully transmit ten messages to a destination device. If the machine enters the one possible unused state(S_0=0, S1=0) then the nano-device will reinitialize the machine. Each simulation involves the transmission of ten packets to a destination device over a simulated channel with a loss rate based on a uniform distribution. It is assumed that packet transmission is the broadcast of encoded biomolecules and the distance between sending and receiving nanodevice is sufficient to permit intercellular communication. As described by Stetter we assume all activation rates are equal and that activation concentrations of input enzymes predominantly reside between 0 and 0.5. The input into the AND and OR unit is a fan-in structure comprising of two input chemicals (Z_{01} and Z_{02}). Assuming the respective activation rates are constant for both Z_{01} and Z_{02} , the input concentration Z_0 can be assumed to be the sum of both concentrations. In the case of an AND gate, two high input concentrations are required to push the system into the upper state of stability. Similarly, the OR gate enters the upper stability point on the occurrence of one high input concentration. Finally the NOT gate is identical to the OR gate except only one input species is considered and output is taken from Z_2.

We also investigate the effect of chemical instability on performance of the chemical machine. The cause of this instability may be due to an unexpected flow of chemicals between sub-compartments. This is simulated by altering the rate at which input chemical species(Z_{0x}) can activate Z_1. This is achieved by offsetting the specific activation rate at which input chemicals affect the active concentration of output Z_1. When this is confined to ability of input species to catalyse output species, equation (3) can be rewritten in the following form:

$$\frac{dZ_1}{dt} = \alpha_{1,1} Z_1 \widetilde{Z}_1 + (\alpha_{1,0} + \varepsilon)(Z_{01} + Z_{02})\widetilde{Z}_1 - \beta_{1,2} Z_2 Z_1, \quad (4)$$

where ε is the magnitude of the disruption. This alters the location of the bistability region of the system and potentially cause the logic unit to function incorrectly. Also, modelling of both chemical noise and channel noise is a realistic assumption that these will be present in a real world system. Fig. 6 shows the output and input chemical concentrations of the logic sub-compartment in Fig 5(b) for the transmission of ten packets over a lossless channel with ε fluctuating randomly $\pm 0.2\,\alpha_{1,0}$. As the input to this gate is S_0 and S_1, it enables the monitoring of the performance of the gate and the current state of the chemical state machine during the simulation. In ideal conditions, the state of the chemical machine continuously transitions between send state to wait state (logic '11' and logic '10'). During the simulation, output of the gate begins to fluctuate and results in an incorrect output at about time=90. Following this, the machine incorrectly transitions to an unused state (S_0=0, S_1=0).

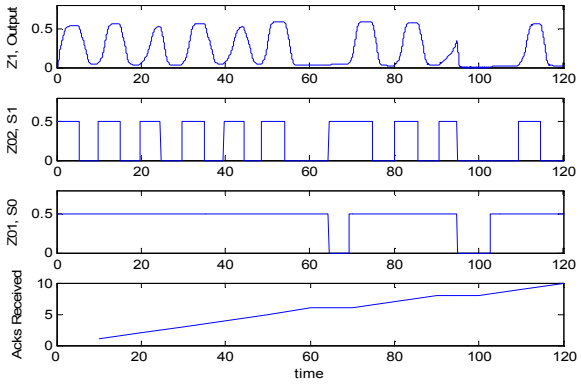

Figure 6. Simulation of AND gate highlighted in Fig. 5 during transmission of ten messages over a lossless channel. Top: trace of output signal Z_1. Second and third from top: Inputs Z_{01}, Z_{02}. Bottom: Acknowledged delivery of messages.

The bottom graph in Fig. 6 shows the effect of this instability on message transmission. The flat regions of this graph indicate message transfer error during simulation and correlates with incorrect machine states (through the simulation run, this resulted in two events). At time=60 the machine state incorrectly remains in a wait state instead of transitioning to resend or transmit state. At time=90, the machine incorrectly transitions to an unused state (S_0=0, S_1=0) after an incorrect output from the sub-compartment shown in fig. 5(b).

The average number of incorrect states due to changes in output activation for a sequence of ten simulations is shown in Fig. 7. Incorrect states begin to occur when the variation of the activation

rate of Z_0 increases above 12% of its stable value. As expected, the number of incorrect state transitions increases significantly beyond this point. In order to investigate the environment for our nano-communication, the performance of the chemical machine was simulated in the presence of both chemical and channel uncertainty. Fig 8 shows the message transfer errors due to channel error rate of 10% and chemical error at $\varepsilon=\pm 0.2\alpha_{1,0}$. The simulation of the chemical machine succeeds in delivering all twenty messages. This is accomplished due to the simplicity of the machine in that a message is only removed from the stack when an acknowledgement is successfully received. However, the simplified protocol also results in time in-efficiency.

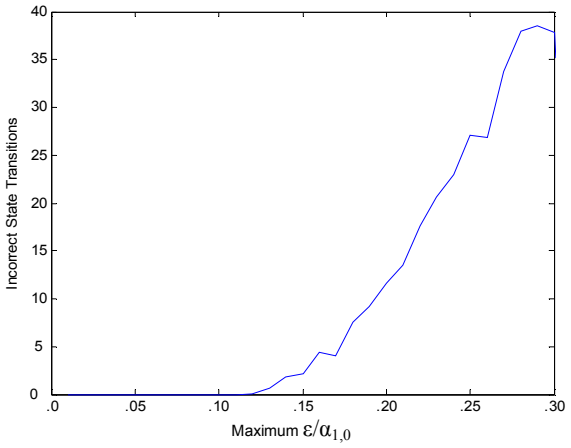

Figure 7. Occurrences of incorrect state transitions relative to the maximum value of $\varepsilon/\alpha_{1,0}$. Failures begin to occur at $\varepsilon/\alpha_{1,0}\approx 0.12$.

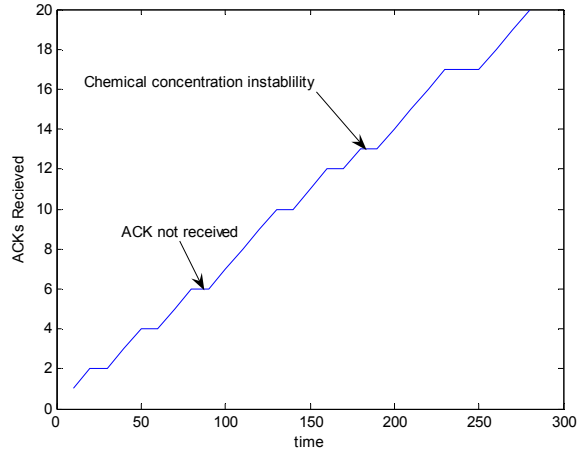

Figure 8. Transmission of 20 messages. Parameters: $\varepsilon=\pm 0.2\alpha_{1,0}$, channel error rate 10%.

Our simulation has illustrated the challenge of communication for nanomachines. Unlike conventional computing devices, where packet loss can be due to lossy channels, in nanocommunication the biomolecule packet can be lost due to both the lossy channel as well as instability in logic operations at the end device. For our future work, we intend to investigate mechanisms to minimise logic operation errors due to chemical instability as well as investigate multiple packet transmission. We will investigate techniques to accomplish windowing and congestion control in a molecular setting. Furthermore, we will also investigate molecular communication interface between the transmitting device and the physical medium and evaluate the usage of current cell-cell signalling techniques such as calcium signalling.

5. CONCLUSION

We have investigated a protocol for reliable molecular based communication between nano-devices, which is based on the reuse of current telecommunications and data communication protocols. The proposed solution involves a bi-stack layer which comprises of compartmentalized nano-logic circuits. The two compartments corresponding to the bi-layers includes Encoding compartment as well as Transmission and Error Recovery compartment. The advantage of our solution is the ability for nanomachines to interface to a cell and control the logical operations for communication. This methodology is ideal for nanomachines which do not require communication capabilities and can control the cell to perform any communication. We have demonstrated the feasibility of this approach through the simulation of a chemical computation based nano-circuit to control transmission and error recovery. Initial simulation results have highlighted the inherent challenges for both chemical and channel instability present in the biological medium. We have shown the effects of both channel noise and unexpected chemical concentration variations on the performance of our transmission and error recovery process.

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A.2 Simulation framework for communication protocols of molecular communication systems

Our previous work in [61] revealed the different characteristics of synthetic biological computation solutions, specifically the contrasts between nucleic acid-based solutions and enzyme-based solutions. It also highlighted the challenge of developing complex communication protocol stacks using these solutions.

In this paper, we match suitable computation solutions from synthetic biology to protocol components in a molecular communication protocol stack. Furthermore, we take into account key design constraints and propose a loosely coupled, condensed communication protocol stack.

Simulation Framework for Communication Protocols of Molecular Communication Systems

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ABSTRACT
In this paper, we describe a protocol stack to enable communication between biological nano scale (*bio-nano*) devices. We identify key design considerations and protocol components and suggest suitable molecular computing mechanisms to create these components. Finally, we introduce a modeling and simulation framework for bio-nano communication networks.

Keywords
Molecular Communication, Molecular Computing, Communication Protocols.

1. INTRODUCTION
Molecular Communication seeks to develop communication mechanisms that use encoded biomolecules as information carriers. Recent experimental results in this area have demonstrated experimentally the creation of a Molecular Communication System [1] and the use of molecular signals for communication between distant bio-nano devices. These advances demonstrate the potential to create biological based communication networks of devices. However, controlled communication on the nano scale is a key challenge in realizing practical applications of bio-nano devices. Just as conventional data networks require communication protocols to function, bio-nano will require the development of similar protocols, implemented using biological based computing techniques. Furthermore, bio-nano devices will require access to the computational capability to implement and control molecular communication mechanisms. We anticipate the bionano devices will be synthetically engineered cells that are functionalized with the required sensors and computing mechanisms to support communication. Alternatively, the bionano device may interface with engineered biological cells, offloading the necessary logic computation required by the communication protocol.

2. Defining Protocols for Molecular Communication

Bionetics'08, November 25-28, 2008, Hyogo, Japan.

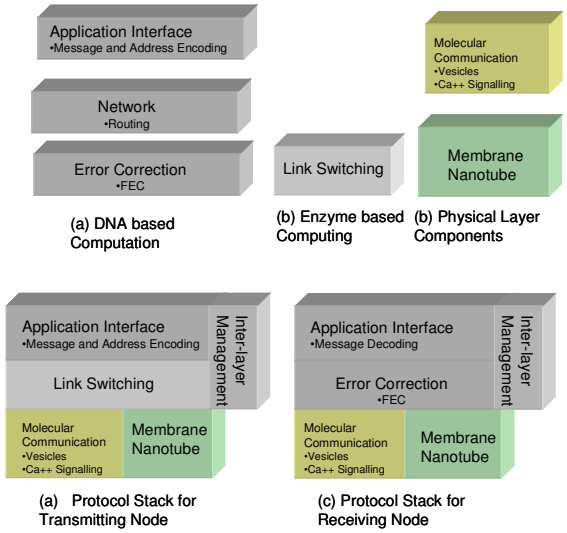

Figure 1. Components of Molecular Communication Protocol Stack.

Inspired by existing communication protocols, we propose a communication protocol stack to enable communication between bio-nano devices. In adopting this approach, the intrinsic characteristics of Molecular Communication relative to standard data communication networks must be considered. For example, Molecular Communication transport is slow, more often via low speed diffusion. Also, bio-nano devices are deployed in harsh biological environments where properties such as pH, temperature and chemical noise are highly variable. These characteristics indicate that high speed transmission and switching found in existing data networks are not achievable. Similarly, connection orientated protocols such as TCP are not suitable due to their associated overhead. Communication is also affected by the potential applications of bio-nano devices which also influences the protocol design where some devices will perform simple sensory functions while others may route data or actuate received commands. Thus we envision two types of data; sensory data (low priority) and command data (high priority). Based on these considerations, we propose a communication protocol stack where components composing the stack depend on device type. This loose association prevents unnecessary complexity for resource limited devices. Fig. 1 illustrates protocol components and suggested compositions for transmitting (sensor) and receiving (actuator) bio-nano devices. Bio-nano devices that only receive

command messages do not require components associated with message encoding and transmission whereas devices that receive command data require error correction capabilities.

Each protocol component is mapped to a suitable biomolecular computing technique depending on its requirements. We consider two molecular computing techniques for this purpose; DNA computing and Enzyme computing. DNA computing is generally considered superior for faster, complex operations and can now be accurately and cheaply engineered to a high degree. Enzyme based computing takes advantage of the bi-stable nature of biochemical enzymatic reactions to create logic operations and is more suitable for simpler computations that require interactions with cytosolic and cell surface components. Fig 1 (a) illustrates the computing techniques selected for each protocol component. Communication protocols can usually be represented as a Finite State Machine which is mapped to either DNA or enzyme computing solution based on location and complexity of the operation. DNA computing techniques are adopted for application interfaces and message encoding while enzyme computing is used for link switching due to its ability to interact with cell surface components. For message encoding, an autonomous DNA encoding automaton based on [3] could be engineered to include the ability to release an encoded biopolymer such as a DNA strand. This automaton process is controlled by a set of DNA "rule" molecules that can be selectively activated by the bio-nano device.

3. Design and Simulation Framework

Bio-nano device research requires the assimilation of technologies and concepts from several research domains. To assist this complex process, we propose a simulation framework to allow researchers investigate, analyze, and design molecular communication systems. Based on our proposed protocol stack, we will develop a set of software and computational components using and combining computational models from systems biology. This approach seeks to extend existing efforts to investigate mechanisms that underpin biological system to create a library of molecular components that can be used to engineer bio-nano systems. We seek to re-use and extend existing design and simulation tools and techniques already emerging in systems biology to enable users of the framework to evaluate cell based communication platforms for molecular communication. Thus the simulation framework can be used by researchers to evaluate designs before progressing to costly and time consuming experimental stage. The high level architecture of the framework is illustrated in Fig 2. The design stage allows the user to design and configure the biological system using a molecular communication toolkit. Components available would relate to environment, bio-nano device, interface mechanisms, and connection type (e.g. gap junction/calcium signaling). The designer stage produces an independent representation of the system that can be simulated using a suitable simulation package.

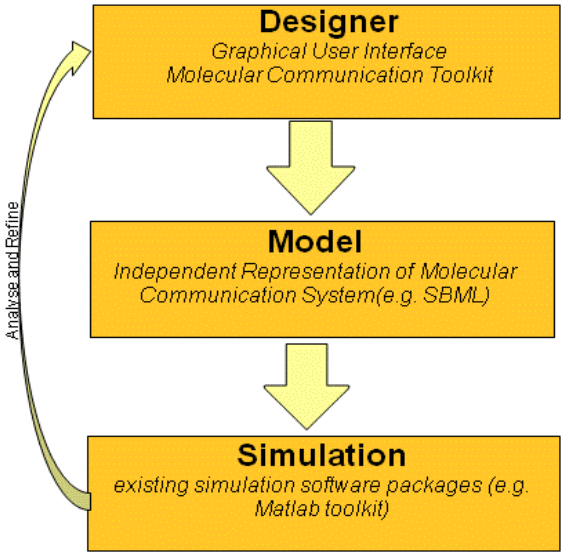

Figure 2: Molecular Communication Simulation Framework

A similar process is adopted in Systems biology research and this research will seek to extend emerging tools and standards such as E-Cell[4] and Systems Biology Markup Language(SBML)[5].

4. Conclusions and Future Work

We are currently investigating protocols for reliable molecular based communication between bio-nano devices based on the reuse of current data communication protocols. Furthermore, we have highlighted the need for modeling simulation tools to support ongoing experimental research in this area. Our future work will include the investigation and evaluation of existing tools and standards to develop a simulation framework for communication between bio-nano devices.

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A.3 Hybrid DNA and enzyme based computing for address encoding, link switching and error correction in molecular communication

In this paper we further extend the concept of a molecular communication protocol stack proposed in [59]. We design a protocol stack for molecular communication nanonetworks which combines two synthetic molecular computing techniques (nucleic acid and enzyme computing), to design a protocol stack for molecular communication networks. The paper describes a solution for several protocol stack functions including biomolecule address encoding, decoding, error correction and link switching mechanisms for molecular communication networks.

Hybrid DNA and Enzyme based Computing for Address Encoding, Link Switching and Error Correction in Molecular Communication

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Abstract. This paper proposes a biological cell-based communication protocol to enable communication between biological nanodevices. Inspired by existing communication network protocols, our solution combines two molecular computing techniques (DNA and enzyme computing), to design a protocol stack for molecular communication networks. Based on computational requirements of each layer of the stack, our solution specifies biomolecule address encoding/decoding, error correction and link switching mechanisms for molecular communication networks.

Keywords: Molecular communication, molecular computing, communication protocols.

1 Introduction

In common with networked computing devices, biological cells have the ability to transmit, receive and process information through signaling networks and signal transduction mechanisms that interact in a complex biochemical system [6][7]. Just as modular silicon components are used to compose digital electronic circuits, the mechanisms that underpin biological systems are now being investigated to create a library of molecular components that can be used to engineer biological based nano (*bio-nano*) scale systems. One good example is Molecular Computing [12], which manipulates biomolecules to engineer biochemical based computing systems. By combining Molecular Computing and Molecular Communication [1], a new research

domain that investigates bio-nano communication, the necessary computing mechanisms can be provided to create communication protocols for bio-nano devices (in the rest of this document, this will be referred to as *nanodevice*). Just as data communication protocols resulted in the rapid growth and ubiquity of networked computing devices and applications, the development of communication protocols for nano-based networks will stimulate groundbreaking future applications of bio-nano devices. The potential applications of these combined technologies are vast, particularly in the medical field where nano-scale devices can perform surgical procedures [14] or ensure accurate drug delivery to specific parts of organs and tissues.

Biological cells contain various components that can play vital roles in networked communication. These include, for example network interfaces (receptors, gap junctions), computing processes (regulatory networks, enzymatic signaling pathways) and memory capabilities (nucleic acids). In this paper, we propose a cell-based communication platform that uses these functional complexities to create protocols necessary for molecular communication networks. Our proposed hybrid solution includes DNA as well as enzyme based computing, where each contributes to specific protocol functions. We will describe how we will re-use protocols from communication networks, and transfer their mechanisms to a cell-based environment. In particular, we will show how our molecular communication protocol stack can support addressing, error correction, and link switching.

The paper is constructed as follows: Section 2 reviews the background of molecular communication and computing. Section 3 investigates protocols for data communication and how we reuse some of these concepts for our proposed protocols for molecular communication. Section 4 presents a simple connectionless communication solution using biological cells as a communication platform for address encoding, error correction, and link switching. Finally, section 5 presents conclusions and future work.

2 Background

2.1 Molecular Communication

Molecular Communication uses encoded molecules as information carriers to engineer biochemical-based communication systems. In [9], Moritani et al define a Molecular Communication Interface that uses vesicles embedded with gap junction proteins to transport message-encoded molecules. The vesicles that embed the information molecules (e.g. this could be represented as metabolites, or small nucleotides) will then be used as signal carriers between the sender and receiver nanodevices. Another form of molecular communication exploits the current calcium signalling that occurs between cells. For example, in [10] Nakano et al showed that distant nanodevices can communicate by encoding information through the frequency and amplitude of inter-cellular calcium waves.

2.2 Molecular Computing

This section will describe two common molecular computing techniques which include DNA and enzyme based computing. A summary and the characteristic differences between the two types of computation are also described.

2.2.1 DNA based Computing

DNA is the universal “information molecule” and has a number of advantages in the computing world, such as encoding information as sequence of biochemical symbols as well as using these symbols to perform computing operations. In [3], Benenson et al present a programmable autonomous finite state automaton consisting entirely of biomolecules. The authors' design consists of a long DNA input molecule that is processed repeatedly by a restriction enzyme, and short DNA “rule” molecules that control the operation of the restriction enzyme. This concept forms the basis for a nanoscale computing machine that diagnoses disease and releases treatment molecules based on several disease-indicating inputs [11]. In [17], Liu et al extended the molecular automaton presented in [11] to design a “DNA-based Killer Automaton” that can release cytotoxic molecules which propagate to neighboring cells via gap junction channels.

2.2.2 Enzyme based Computing

Markevich et al [4] created a bistable switch using a cell-based Kinase-Phophatase signaling cascade (MAPK) that is highly conserved in eukaryotic cells. In doing so, the author demonstrates the use of ultra-sensitive cell-based enzyme signaling pathways to perform digital logic computation. Similarly, in [5] Stetter et al uses the bistable nature of biochemical enzymatic reactions to create a reusable, “easy to engineer” architecture that forms the basis of several Boolean logic functions such as AND, and OR gates. This small enzyme-based circuit can act as a sub-component in composing more complex functions.

There are a number of differences between the two types of cell-based computing, where each has certain disadvantages and advantages with respect to computing for communication protocols. Firstly, the computational complexity and speed associated with DNA computing is, as yet, not attainable using enzyme based computing [16]. Also, the parameter characterization effort required to achieve enzyme computing increases dramatically relative to circuit complexity [13]. This makes enzyme computing more suitable for relatively simpler circuits that require short computation time. On the other hand, DNA-based computing can support larger computing requirements. The other difference between enzyme and DNA computing is that enzymatic reactions are intrinsic in cytosolic cell signaling pathways [7]. Therefore, this allows closer interaction with cell membrane components such as receptors and gap junctions. This makes it particularly suitable to simpler, responsive computing involving extra-cellular input and output.

3 Defining Protocols for Molecular Communication

In this section we will first describe the core characteristics of communication network protocols, and how these protocols will be re-used to support nanodevices.

3.1 Communication Network protocols

Communication networks consist of protocols that exhibit the following properties; access mechanisms to physical communication interfaces, encoding and addressing mechanisms, error detection/correction techniques, and routing of packets between connected nodes. Physical interface controllers provide connection to physical transmission media and include mechanisms such as modulation and channel coding. The link layer functions manage access to the underlying physical layer, while flow control and acknowledgment mechanisms are usually implemented in higher layer protocols such as TCP. Communication can be connectionless or connection-oriented, where connectionless communication have lower data overhead, and are suitable for energy efficient networks such as wireless sensor networks. Another common protocol used in communication network is error correction, where techniques such as Forward Error Correction (FEC) can ensure that end devices can recover from any data corruption incurred during transmission. One approach is through inclusion of redundancy in channel encoding process.

3.2 Protocols for Molecular Communication

As described earlier, our intention is to be able to re-use protocols from conventional communication networks for molecular communication. Fig. 2 illustrates the components of our protocol stack and the protocols for different operations of the nanodevice (e.g. Transmitting node, Receiving Node, Intermediate Routing Node). Our approach is based on interconnection of loose protocol components, where each component is performed by a specific molecular computing technique. The reason that we have not embed all components into a generic protocol stack, is to prevent unnecessary increase in computational complexity. Although, the components of each layer is mapped from conventional protocols used in communication networks, the layers of our protocol stack is re-organised to suit a number of characteristics found in molecular communication. For example, propagation of information in molecular communication is typically characterized as low speed and in an environment where the interconnecting links between nano devices use biological signaling mechanisms that are highly variable compared to standard communication networks [1][2]. These characteristics have repercussions for the design of protocols of molecular communication systems. Slow diffusion-based processes do not support the creation of high-speed switching functions common in conventional network devices that will require complex queuing mechanisms for packets. At the same time, due to high variability and harsh biological environment, the use of acknowledgements and retransmission of messages in the event of loss or corrupt packets may not lead to improved performance.

We anticipate two types of information transmissions used in molecular communications, which includes sensory data (data collected from nanodevices) and command data (instructions for nanodevices). Therefore, the transmission mechanism and protocols to be used will be highly dependent on the nature of the information. For example, for sensor data, we may use single paths with UDP-like transmission with no error correction. However, command information or high priority sensor data will be transmitted through redundant paths with error correction capabilities (e.g. FEC).

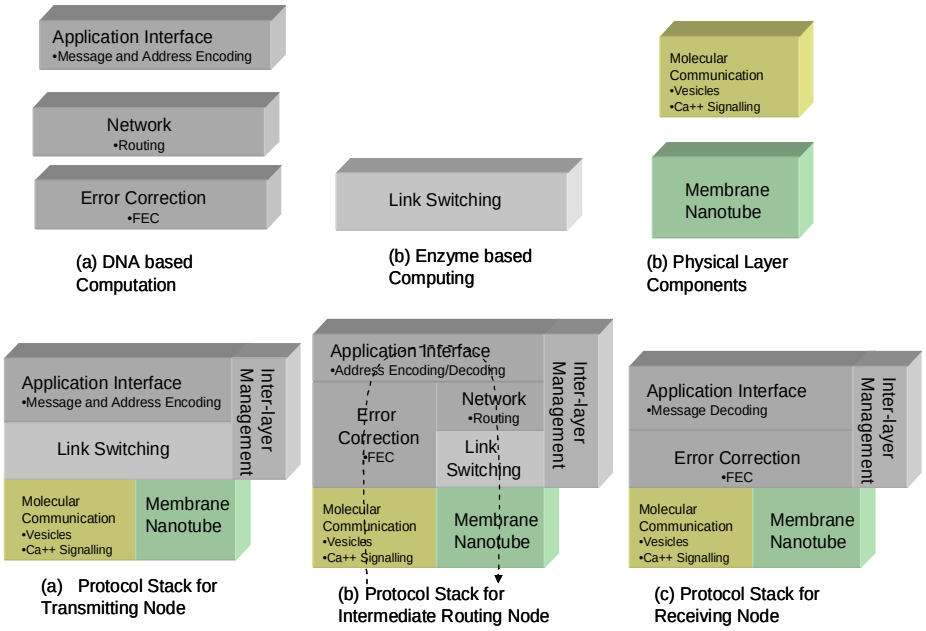


Fig. 1. Molecular Communication protocol stack.

Since protocols can usually be defined through a Finite State Machine (FSM), we adopt a nano-logic circuit that is translated from a FSM to represent the different types of protocols. We then map the specific protocol to either DNA or enzyme based computing. Since each technique has its own characteristics, we apply and select the right techniques based on two factors which includes, (i) the sequence of operation for the protocol, and (ii) complexity of computation required for the protocol. The DNA based computing is used for *Application Interface*, *Network*, and *Error Correction* layers, while the enzyme based computing is used for the *Link Switching* layer. The Application Interface, Network, and Error Correction layers will require higher complexity computation and is usually not required to be time sensitive. Such computations will include FEC, addressing, and information encoding/decoding. Enzyme based computing, due to its limited time requirement, is most suitable in performing small size logic circuit with high-speed computation. Therefore, this is most ideal for switching of information biomolecules between the links. The underlying physical layer can be based on solutions by [1] [10] for molecular communication, where the molecular communication can be guided through membrane nanotubes [19]. We se-

lect membrane nanotubes as a physical layer communication mechanism between cells, essentially providing the guided channels interconnecting each node in the bio-nano network. Unlike intercellular communication mechanisms that broadcast chemical signals to all neighboring cells via intercellular space, these nano-tubular structures can create a network of communication links between distinct cells that can support intercellular transfer of cytosolic molecules, vesicles and organelles. A notable work is by Önfelt et al who demonstrated a membrane network that transports tagged vesicles from cell to cell [19]. Therefore, the membrane nanotubes could be used in conjunction with a suitable molecular communication mechanism such as [9] that uses vesicles to transport message molecules or [10] to guide modulated calcium "waves" from sending cell to receiving cell.

In between the two layers will be the *Inter-layer protocol management*, which will coordinate the different computation of each layer of the protocols and the location where this will happen in the cell. Fig. 2 illustrates our solution that combines a subset of our proposed protocol to support transmission on a single link.

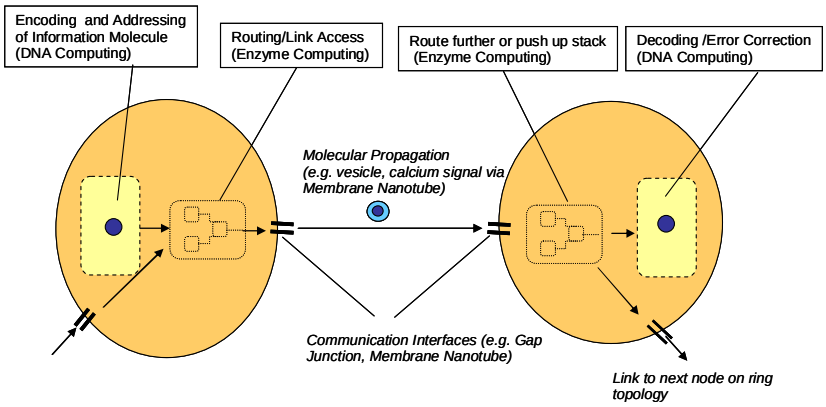


Fig 2. Mechanism of transmission for single link molecular communication

The flow of operation between the layers is as follows. For the Transmitting Node (Fig. 1(a)), the Application layer Interface will perform the message encoding for the information biomolecules. The encoded biomolecule is then further encoded with the specific address of the intended destination using an address table. In our proposed protocol stack, we have left our application layer open, where the cells can interface to a physical device or we can have artificial cells with embedded functionalities (e.g. the cell also acts as the device). Once the encoding process is performed, the information biomolecule is ready for transmission and submitted to the Link Switching layer, which selects the correct gap junction for transmission. In the Intermediate Routing Nodes case (Fig. 1(b)), when the biomolecule is received by the cell, the error correction is first performed on the biomolecule. This is then followed by the address decoding and encoding process based on the routing table for the next node. Once this is performed, the link switching operation follows and transmits the biomolecule to the underlying link. Once the information biomolecule is received at the receiving device (Fig. 1(c)), the information biomolecule is once again passed through the Error Cor-

rection layer to perform any necessary error correction, which is then followed by the message decoding at the Application Interface layer.

4 Proposed Solution

In this section, we will describe the molecular computing operations for information encoding and addressing, link switching, as well as error correction.

4.1 Encoding and Addressing

Fig. 3(a) illustrates the encoding process. Similar to the model proposed by Liu et al in [17], our solution uses Benenson's and Shapiro's work in [3] to create a DNA-based automaton that produces a *single strand DNA* (*ssDNA*) message molecules for intercellular communication. Each ssDNA message is encoded as a unique sequence of nucleotide bases as demonstrated in [4]. For simplicity, only three addressable nano-device nodes are considered and each encoded ssDNA message is 'framed' to include addressing information.

Fig. 3(a) illustrates how nucleotide encoded messages are assembled in sequence of long input double stranded DNA message molecule with each message separated by a 'spacer' sequence. The upper leftmost "sticky end" represents the current state of the machine. During the address encoding process, the DNA message molecule is cut by a restriction enzyme, which releases the leftmost segment of the molecule. Thus the <*address*, *message*> pairing represented by the current state of the encoding automaton is released as an ssDNA segment through the restriction process. Fig. 3(b) illustrates how each address state and transition corresponds to actual encoded message molecule. Each state transition is enacted by a corresponding DNA "rule" molecule and enzyme complex that cleaves the corresponding nucleotide sequences. A key characteristic of address encoding is the precise cleaving of input message molecule that encodes or "frames" the message.

Fig. 4 illustrates a rule execution transition from Address 2 to Address 3. Each rule molecule has a recognition site to which a restriction enzyme can bind. As described earlier, the number of nucleotide bases between the restriction enzyme and the sticky end of the rule molecule determines the precise locations of the message molecule cleave. In this example, the restriction enzyme complex combines with the message molecule and cuts at fourteen nucleotides on the top and twenty-one nucleotides at the bottom. The resulting new sticky end reveals the next state of the automaton. More importantly, the segment that is cut away separates into two ssDNA molecules. The lower ssDNA molecule indicated in Fig. 4 is the encoded message molecule with its rightmost end complementary to the new sticky end of the DNA message molecule.

Similar to techniques used in [17] and [11], the nanodevice can control computation by releasing molecules (e.g. mRNA) that selectively activate DNA *"rule"* molecules. The results of the computation can provide input to other parallel computational functions, which was proposed in [3]. In our solution, the cleaved ssDNA message molecules are released into the cytosol and provide the input to the molecular interface control function of the network layer. Theoretically, this mechanism can be ex-

tended to encode a multitude of unique address locations and any number of messages during computation.

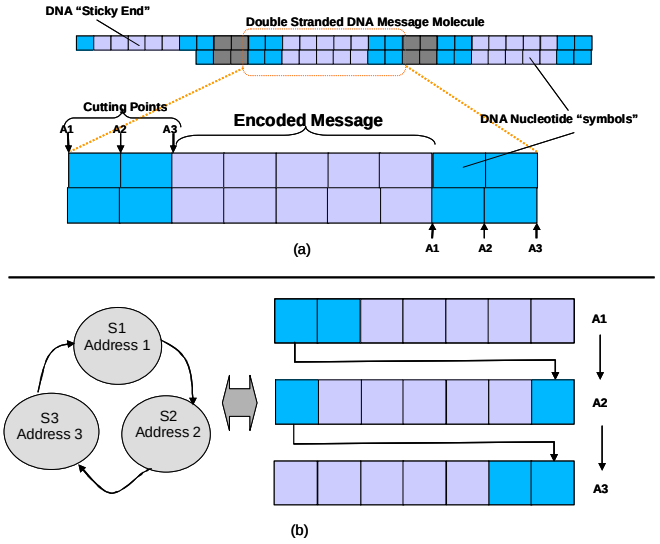


Fig. 3. (a) Double Stranded DNA message molecule indicating restriction cut points for address encoding, (b) State representation of address encoding transitions.

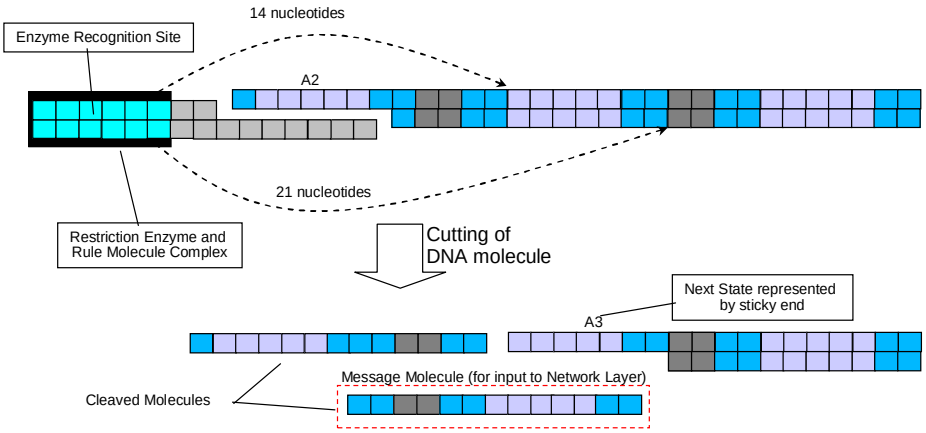


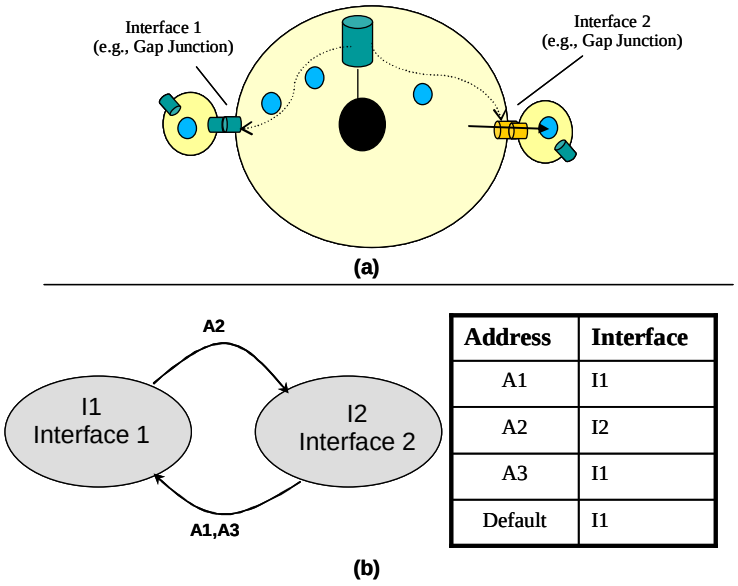
Fig. 4. Mechanism of State Transition from Address 2 to Address 3 using Benenson's Molecular Automata [3].

4.2 Molecular Interface Control

As described earlier, the operation of our molecular communication is through a membrane nanotube network. Fig. 5 illustrates a cell with two distinct molecular

communication interfaces (e.g. distinct gap junctions). Each addressable location is switched through the corresponding interface according to the addressing state diagram shown in Fig. 5 (b). For communication involving the transfer of message molecules through gap junctions, our solution is based on results in [18] which demonstrate the diffusion of synthetic oligonucleotides through gap junction channels. In our case, instead of oligonucleotides, we diffuse our encoded ssDNA from the previous section.

In this study, interface selection is achieved using the "real world" implementation of the logical recurrent architecture as described by Stetter et al in [5]. The switching circuit releases/alters a corresponding chemical signal that "switches" the ssDNA to the correct interface. In the case of gap junction interfaces, the output of the enzyme-based circuit will control the permeability of gap junction channels. Gap junction permeability is affected by the connexin phosphorylation [10] via specific concentration of phosphorylation reagents.



Address	Interface
A1	I1
A2	I2
A3	I1
Default	I1

Fig. 5. (a) Schematic diagram of cell with two distinct molecular communication interfaces, (b) Address/Interface state diagram and switching table.

Thus Stetter's circuit can be used to effectively switch on and off each molecular communication interface by controlling the degree of phosphorylation of gap junction connexins. This in turn will allow the ssDNA to be pushed through only a single link (or multiple links if multicasting is used). Using this technique, several communication links can be controlled simultaneously via compartmentalized enzymatic functions[8]. The Inter-layer protocol we will be responsible for triggering the enzymatic computation, once the operation from the Application Interface layer is complete (the operation of this mechanism is subject to future work).

4.3 DNA Decoding and Forward Error Correction

As already stated, prioritized messages require error detection and correction. Invariably, errors will occur in the encoding and transmission process of ssDNA molecules due to the imprecise nature of the associated complex biochemical reactions [15]. By including redundancy in the encoding process, error correction mechanisms can be incorporated into the decoding process. Our solution combines the nucleotide redundancy concept presented in [16] with DNA automata design in [11] to create an autonomous error correction mechanism. In our proposed technique, each ssDNA molecule is composed of several repeated, identical nucleotide sequences.

In [11] Benenson uses "protector strands" to control the operation of an enzyme based state machine by separating the constituent DNA strands of message molecules (see Fig. 6). In our solution, the protector strands are designed to have a strong affinity for a specific received ssDNA. The ssDNA molecules cause the corresponding protector strand to separate from the transition strand and hybridize with the message molecule allowing the formation, and thus activation, of a double stranded transition molecule (a similar mechanism to the encoding process). The resulting transition molecule and restriction enzyme complex cleaves the corresponding decoding DNA molecule and releases the decoded DNA molecule (in Fig. 6, this is represented as the end DNA hairpin) with no errors. Our assumption of this approach is mainly for finite instruction messages, where our end device will contain as many Decoding DNA molecule as the number of possible instructions. Hybridization can also occur even though both the protector strand and the received ssDNA molecule are not exactly complementary.

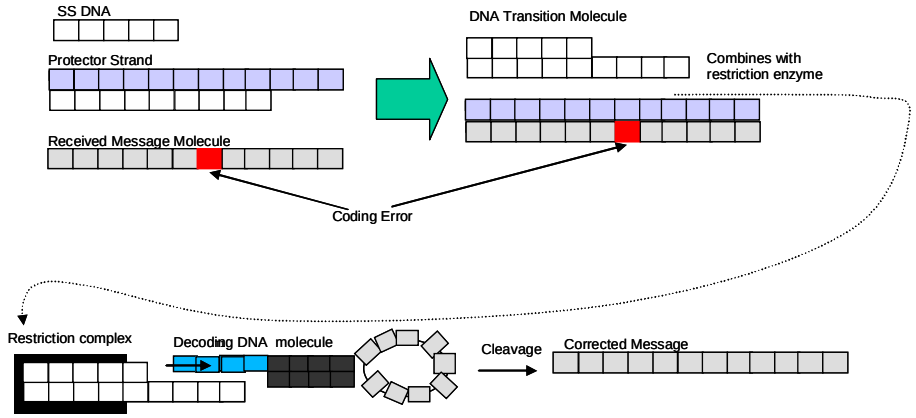


Fig. 6. Forward Error Correction Mechanism

5 Conclusion and Future Work

Inspired by protocols for communication networks, we have presented a molecular communication protocol stack that successfully combines molecular computing and

molecular communication techniques. We describe how the core characteristics of communication network protocols are re-used to design bio-nano device communication protocols. Our proposed protocol stack presents the address encoding/decoding, link switching, and error correction functions that are developed using molecular computing techniques. The solution demonstrates the necessity of matching the characteristics of each molecular computing technique to the computational requirements of each layer of the proposed protocol stack. Our future work will investigate the feasibility of our design initially through simulation of chemical circuits for molecule encoding/decoding, link switching and error correction.

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A.4 Synthetic protocols for nano sensor transmitting platforms using enzyme and DNA based computing

In our previous published work we highlighted the potential of the biological cell as a communication platform for molecular communication [61]. This paper develops this concept by presenting a biological cell-based communication platform using synthetic molecular computing techniques. Two protocol stack solutions are presented and, for each solution, we take care to use cell-based synthetic biology techniques which have been proven experimentally. Furthermore, we begin to use accepted and curated models for MAPK enzyme and calcium ion signalling to design, characterise, and simulate the performance of the protocol stack.

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Synthetic protocols for nano sensor transmitting platforms using enzyme and DNA based computing

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ABSTRACT

The ability to create communication networks of biological nanoscale devices has the potential to open up new opportunities and applications, particularly in areas such as health care and information processing. Inspired by recent developments in molecular communication and biomolecular computing, we present in this paper a biological cell based molecular communication transmitting platform using synthetic molecular computing techniques. We investigated two protocol solutions which include DNA based computing coupled with viral particles and enzyme based computing coupled with calcium signaling. Each of these solutions is designed for different applications and environments. For each of these approaches we demonstrate how elements from various layers in the communication stack are developed using the molecular computing mechanisms. Simulation results are also presented to illustrate the functionality and performance of each solution.

1. Introduction

In recent years, advancements in nanotechnology have attracted tremendous attention, particularly in the field of medicine. These developments have ranged from advances in materials for nano devices, to improved capabilities (e.g. self-assembling) as well as applications (e.g. diagnosis, drug delivery). While these developments have increased application opportunities for nano devices, a specific aspect that is still in its infancy is communication capabilities. This has led to new research opportunities of nanoscale communication and networking, which is being currently pursued by communication network researchers [2]. Improving the communication capabilities of nano devices can greatly impact not only the current applications of nano devices, but also open new opportunities for uses of nanotechnology (e.g. networked nano sensors). Molecular communication is one research field that has investigated communication capabilities between nano devices, particularly in biological environments [29,2,16]. Over the years, development of communication systems has evolved from fixed infrastructure to wireless communication for mobile and sensor devices, where the design paradigms have remained the same. Similar to design paradigms used in conventional communication systems, molecular communication aims to encode information into biomolecules and diffuse this through the biological medium to nano device receivers, which in turn will decode this information.

However, developing molecular communication systems with the same design paradigms of conventional communication systems brings new challenges. Firstly, nano machines, unlike computing devices, have limited capabilities for incorporating dedicated physical communication systems. Therefore, any communication mechanism must be developed using components within biological environment. Secondly, information would have to be transformed into biomolecules in order to be passed to the underlying biological environments. Thirdly, biological environments as a physical media are very different in nature to conventional physical communication media, where propagation of molecules is usually based on diffusion and are very slow. Therefore, acknowledgements

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of messages may not be suitable in ensuring the reliability of message transmissions. Based on these challenges, communication protocols designed for nano devices will need to be modular, flexible and highly dependent on applications. In this paper, we propose solutions for communication protocols for nano devices, based on the original TCP/IP protocol stack design paradigm. While various attempts have been made to improve communication network performance by adopting mechanisms from biology, we have taken an opposite approach [9,10]. The functionalities of each layer of the protocol stack are realized through existing molecular computing approaches [32,30]. In particular, we focus on two molecular computing approaches, which includes DNA and enzyme based computing solutions. Each approach is tailored to suit different physical layer molecular communication mechanism, which includes calcium signaling as well as viral particle biomolecule approach. We have designed the two protocols as a communication platform that can target various nano device applications, but mainly focusing on synthetic nano sensors.

The paper is organized as follows: Section 2 presents related work on molecular communications and molecular computing. Section 3 presents a high level overview of applications of molecular communication network that can exploit our proposed protocol design. Section 4 presents the enzyme based computing approach that is coupled with calcium signaling, while Section 5 presents the DNA computing approach that is coupled with viral particle biomolecules. Section 6 presents the discussion, and finally, Section 7 presents the conclusion.

2. Related work

The related work is divided into two sections, which includes current research work in molecular communications and molecular computing.

2.1. Molecular communication

In our previous work [43] we reviewed molecular communication as a communication mechanism for biological nano and BioMEMS (Biological Microelectromechanical Systems) devices. In [29], Nakano et al. showed through both experiments and simulation, that distant nano devices can communicate by encoding information through the temporal and spatial properties of intercellular calcium waves. Intercellular waves are shown to propagate through a multicellular environment via connexons, where two connexons form gap junctions between the cells allowing exchange of molecules and ions. In [28], Moritani et al. define a molecular communication interface that uses vesicles embedded with gap junction proteins to transport encoded molecules. The vesicles encapsulate the information molecules (e.g. this could be represented as metabolites, or small nucleotides) and are used as signal carriers between the sender and receiver nano devices. In [8], Bush et al. investigates the impact of topology sensitivity, information capacity, and latency on carbon nanotube (CNT) networks for bio-nano applications, drawing comparisons to cell based microtubule structures. The author examines effects such as isotropy and persistence length on CNT performance and also proposes autocorrelation and persistence area as design metrics for CNT bio-nano networks. Eckford [11] addresses molecular communication from an information theoretic context, formulating an abstract model for molecular communication applicable to both communication and information theorists. In all these research work, the focus of investigation has largely been at the physical layer, which can be complementary to the solution of upper layer protocols that we propose in this paper.

2.2. Biomolecular computing

Biomolecular computing (from here on we will just use the term *molecular computing*) uses biomolecules, biochemistry and molecular biology to perform computation. In the context of the solution proposed in this paper, molecular computing provides a programmable mechanism to implement communication protocols in bio-nano devices. This section examines two common biomolecular computing methods; DNA and enzyme based computing. A summary and the characteristic differences between the two types of computation are also described.

2.2.1. DNA based computing

DNA molecules have a number of advantages for biomolecular computation, such as encoding information as sequence of biochemical symbols as well as using these symbols to perform computing operations. In [33], Rinaudo et al. use RNA interference (RNAi) in biological cells to implement generic Boolean logic. This solution forms the basis for a bio-nano device that can respond to and make decisions based on incoming, extracellular inputs. This work extends Benenson et al.'s work in [5] that investigated a programmable autonomous finite state automaton consisting entirely of biomolecules. The autonomous finite state automaton consists of a long double stranded DNA input molecule that is processed repeatedly by a restriction nuclease *FokI*, where short DNA "rule" molecules control the operation of *FolkI*. These concepts can form the core of programmable bio-nano devices that can be designed to carry out several tasks such as *in vivo* disease detection and the release of treatment based on several disease-indicating inputs.

2.2.2. Enzyme based computing

In [38], Stetter et al. uses the bistable nature of biochemical enzymatic reactions to create a reusable architecture that forms the basis of several Boolean logic functions such as AND, and OR gates. This small enzyme based circuit can act as a sub-component in composing more complex functions. A key requirement of enzyme based Boolean functions is the sinusoidal, "*switch-like*" response to inputs. For example, in [26] Markevich et al. describe a bistable switch using a cell based Kinase–Phosphatase signaling cascade that is highly conserved in eukaryotic cells. In doing so, the authors demonstrate the use of ultrasensitive cell based enzyme signaling pathways to perform digital logic computation. Similarly, in [32] Prasanna de Silva et al. demonstrate the potential for

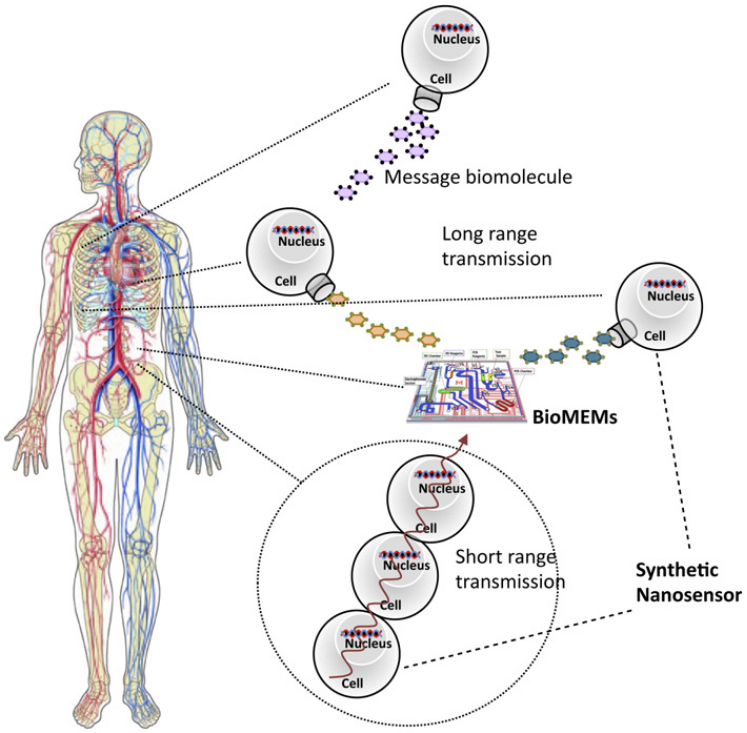

Fig. 1. Nanoscale communication network interconnecting nano sensors.

modularising several logic operations into one enzyme based reaction pathway using molecular and enzyme reactions. These approaches could be orchestrated to functionalize the computation required to implement protocols for nano devices.

A number of differences between the two methods of molecular computing is apparent and each method has certain disadvantages and advantages with respect to computing for communication protocols. The computational complexity associated with DNA computing is, as yet, not attainable using enzyme based computing [38]. Also, the parameter characterization effort required to achieve enzyme computing increases dramatically relative to circuit complexity [30]. This makes enzyme computing more suitable for relatively simpler circuits that require short computation time. Conversely, DNA based computing can support larger computing requirements.

3. Application of molecular communication

An illustration of the use of molecular communication in a biological environment is shown in Fig. 1. In this example, we can see a number of nano sensors embedded within the human body at different locations. These synthetic nano sensors (for the rest of this paper, we will refer to this as *nano sensor*) can detect abnormalities in the surrounding tissue and release information to distant sinks. Such nano sensors may target high-risk regions of tissues in specific organs that require information to be updated in a timely manner. The information from the nano sensors can be transmitted to a single point, which could be a BioMEMS device. The BioMEMS device could in turn transmit collected information to external devices to be further analyzed.

The nano sensor could be a simple switch automaton that is embedded into a cell and release very small information that is propagated to the sink. For example, a biological monitoring application measuring toxicity levels in a tissue may require swift actuation of response should the toxicity threshold be breached. The nano sensor may also embed an automaton machinery that performs limited computation and produces biomolecule based information which is diffused into the environment. Our approach is to view the biological cell as a communication platform and, by manipulating the biochemical sensing and computational mechanisms, implement communication protocols. The communication protocol that we considered in our proposed solution includes reliability control, addressing, and information encoding (only in the case of enzyme computing). The choice for optimum computation mechanism is dependent on application type [43]. Similarly, the choice of physical signaling mechanism is also application dependent.

4. Enzyme based protocols

In this section we present the enzyme based protocols supporting nano sensors that communicate using calcium ions (Ca^{2+}) at the physical layer. The protocol structure is illustrated in Fig. 2.

The enzyme based protocols are designed for simple sensors with minimal functionalities and can transmit to sinks at short effective communication radius. In particular the communication protocol is suitable for sensors that require rapid response time. Fig. 3 illustrates the operation of the enzyme protocols used to control Ca^{2+} signaling. The following subsections will describe the enzyme protocols and the Ca^{2+} signaling in detail.

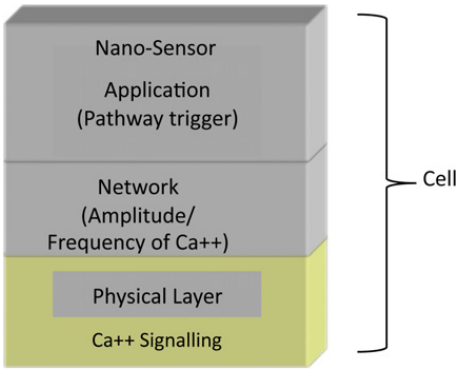

Fig. 2. Protocols using enzyme based computing.

4.1. MAPK based logic gates

The enzyme based protocols utilize the pathway reactions within the cytosol. Although there are various methodologies to enable this form of computation, we mainly focus on an approach proposed by Sauro et al. in [34], whereby the Mitogen-Activated Protein Kinase (MAPK) signaling pathway is used as a "plug-and-play" component. MAPK pathways are a family of signaling pathways which consist of a cascade of three enzymes that act in series in response to extracellular stimuli. The MAPK signaling pathway is one of the more well known pathways, where numerous studies have been performed, including bio-inspired approaches that use MAPK signaling models for enhancing network security [22].

We use the MAPK pathway to execute the biochemical based computations required to implement nano sensor application. The effect is to produce an activated protein or enzyme in the cytosol that represents the detectable event. Using receptors coupled with enzyme cascades has several advantages: (1) each receptor and ligand can be uniquely complimentary providing a high degree of specificity, (2) enzyme cascades can produce amplification of extracellular signal [32], (3) feedback loops in enzyme cascades [44] can produce a constant and stable cytosolic response to a fluctuating external signal, and (4) the ultrasensitive (switch-like) response of enzyme pathways to input stimulus means they can be used as components for biological computation. Ultrasensitive input/response curves generally can be fitted to Hill equation model with a Hill coefficient greater than 1 [18], which would indicate suitability of the switching operation.

The advantage of this approach is that, irrespective of extracellular conditions, the homeostatic nature of the receptor response means that the resulting cytosolic chemical response is stabilized within a small range of concentration levels. In our application, these concentration levels represent logic input states, which in turn represents the protocols. Such protocols will be responsible for encoding the information and triggering the necessary Ca^{2+} signaling.

4.2. Ca^{2+} encoding using Inositol Triphosphate (IP3) manipulation

Calcium signaling is a common short range cellular signaling mechanism in biological systems with information encoded in the temporal and spatial properties of Ca^{2+} concentrations [6,40]. Several authors have demonstrated the operation of a communication channel using the calcium ion transmission as a physical communication mechanism [41,17]. In particular, Nakano et al. [29] investigated the controlled transmission of a calcium wave through a biological cell based channel, demonstrating the viability of calcium ions as a mechanism for molecular communication. Intercellular calcium waves provide an effective short range communication mechanism capable of travelling distances in the order of micrometers in relatively short time span [23]. However, such distances require the ability of channel forming cells to propagate the calcium wave using their own calcium sources.

In [15], Goldbetter et al. developed a model for Ca^{2+} release that can be modulated by phosphorilated proteins. Similarly, in [36], Schuster et al. explain that cytosolic proteins play a part in frequency and amplitude regulation of intracellular calcium oscillations. Taking the computational model proposed in [15], cytosolic Ca^{2+} is modulated by the Inositol Triphosphate (IP3) concentration in the cell represented by IP3 saturation function. By promoting IP3 release in the cell through internal activation of the IP3 pathway, it is possible to manipulate the concentration of cytosolic IP3 in the cell. For example, the formation of IP3 relies on the presence of active PI-phospholipases (PLC) enzymes [35]. A common element in all PLC activations involves the activation of kinases that phosphorylate the PLCs. Another possible technique, described in [39], is to modulate cytosolic Ca^{2+}through phosphorylation of intracellular IP3 receptors on the endoplasmic reticulum using protein kinases. Our solution proposes a biological state machine that activates the kinases that control PLC and, in turn, control IP3 and released Ca^{2+} concentration level. Using the Goldbetter model in [15], this means the IP3 saturation function is parameterized by the output of an encoding circuit. The following case study explores through

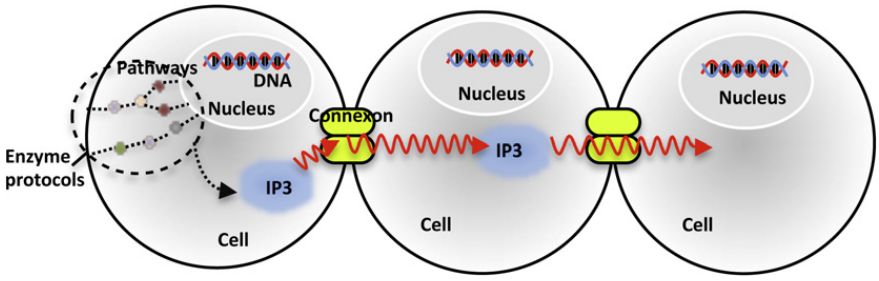

Fig. 3. Illustration of enzyme protocol controlling Ca^{2+} signaling.

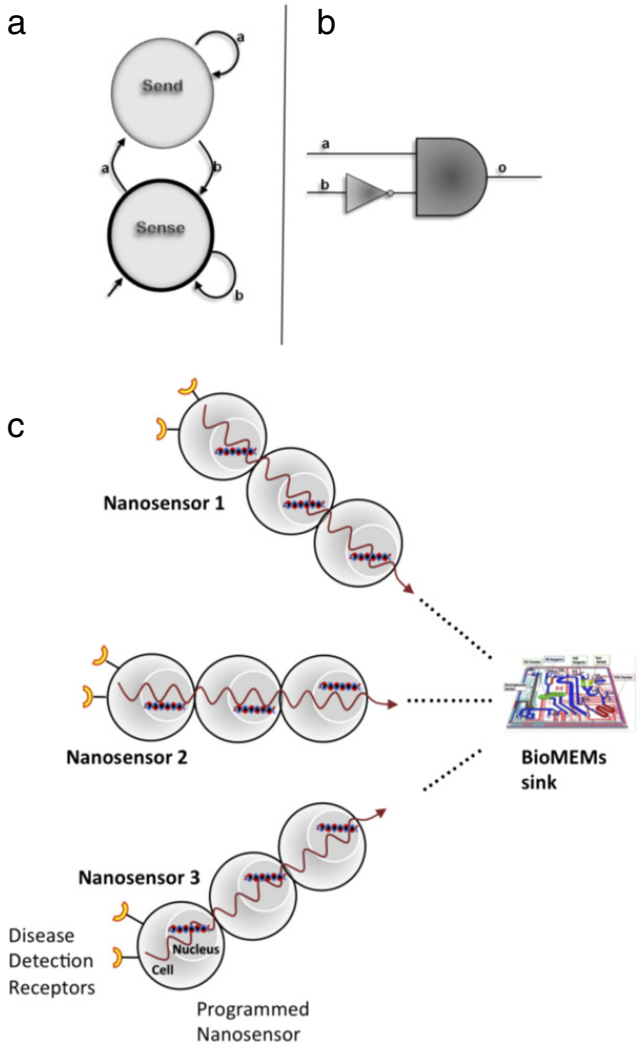

Fig. 4. (a) Bio-nano sensor state diagram. (b) Corresponding logic diagram. (c) Case study scenario of 3 nano sensors that sends signals to BioMEMS sink.

simulation, a cell based nano sensor that uses enzyme based protocol to encode Ca^{2+} signals in response to the detection of extracellular toxin.

4.3. Case study—disease detection

To investigate the viability of this approach, we simulated a cell based nano sensor network in a common multicellular channel using intercellular Ca^{2+} signaling as the physical communication mechanism. Fig. 4(c) illustrates our case study, where there are three nano sensors that can transmit information to a BioMEMS sink. Each nano sensor is engineered to detect biochemical indicators of a specific human disease, through engineered receptors on the cell surface. We focus our case study on implementing the application and network protocol components of the protocol stack in Fig. 2 and simulate a simple transmission state machine in Fig. 4(a) to represent the application function of the nano sensors.

In Fig. 4(a), each sensor by default is initialized to a *Sense* state. The detection of a target substance causes the transition to *Send* state resulting in the encoding and transmission of Ca^{2+} signal. The transitions *a* and *b* correspond

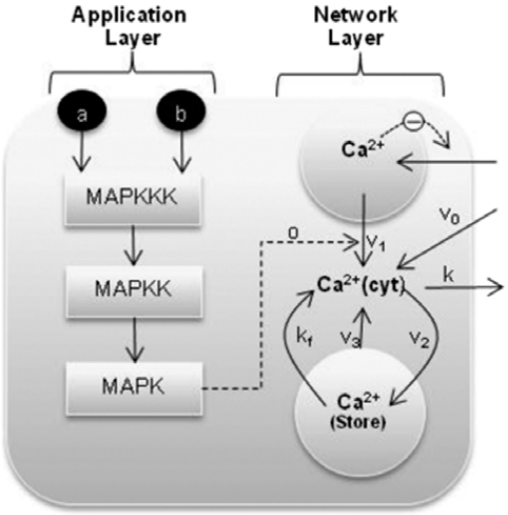

Fig. 5. A schematic representation of enzyme based bio-nano sensor. The MAPK signaling pathway represents the application layer, while the Ca^{2+} signaling represents the network layer.

to occurrences of specific input concentration, which may be triggered from engineered receptors described earlier. The reset signal, represented by transition *b*, results in transition to Sense state, which can be received from the BioMEMS device of Fig. 4(c) (in this paper, we do not consider the receiving mechanisms). A schematic representation of an enzyme based bio-nano senor is shown in Fig. 5. The following sections describe the design of the application and network layer components.

4.3.1. Application layer

We use the ultrasensitive MAPK enzyme cascade model from [18] to functionalize the Boolean logic operation. For simulation purposes, a curate System Biology Markup Language (SBML) model from *BioModels* database [24] was imported into MATLAB using the SBML toolbox [20]. Fig. 6(a) shows the mapping of the MAPK model to the logic required to operate the nano sensor in Fig. 4. The ultrasensitive response of this circuit is apparent in Fig. 6(b), which shows output for a range of input concentrations and highlights its suitability for Boolean logic applications. The next step is to characterize the operation of the enzyme pathway as a logic function.

The output can be expressed as a function of both inputs, and, in this case, input signals *a* and *b* correspond to the promoter and inhibitor of the first layer of the MAPK enzyme cascade. Each input state, $s = (a, b)$, to the enzyme logic operation can be represented logically as a set of possible inputs, $S = \{(a_0, b_0), (a_0, b_1), (a_1, b_0), (a_1, b_1)\}$ where a_0 and a_1 correspond to chemical concentrations that represent logic 0 and 1, respectively. A similar scheme is used for input *b*. We model input concentration values as a normal distribution, $a_0 \sim N(\mu_{a0}, \sigma_a^2)$, where μ_{a0} is the expected value of a_0 and σ_a^2 is the concentration variance of *a*. Similar expressions are used for other input values. The input values are re-sampled every five seconds to simulate ongoing effects of noise. The output takes the form of a bivariate normal distribution and we assume both inputs *a* and *b* are uncorrelated for this simulation.

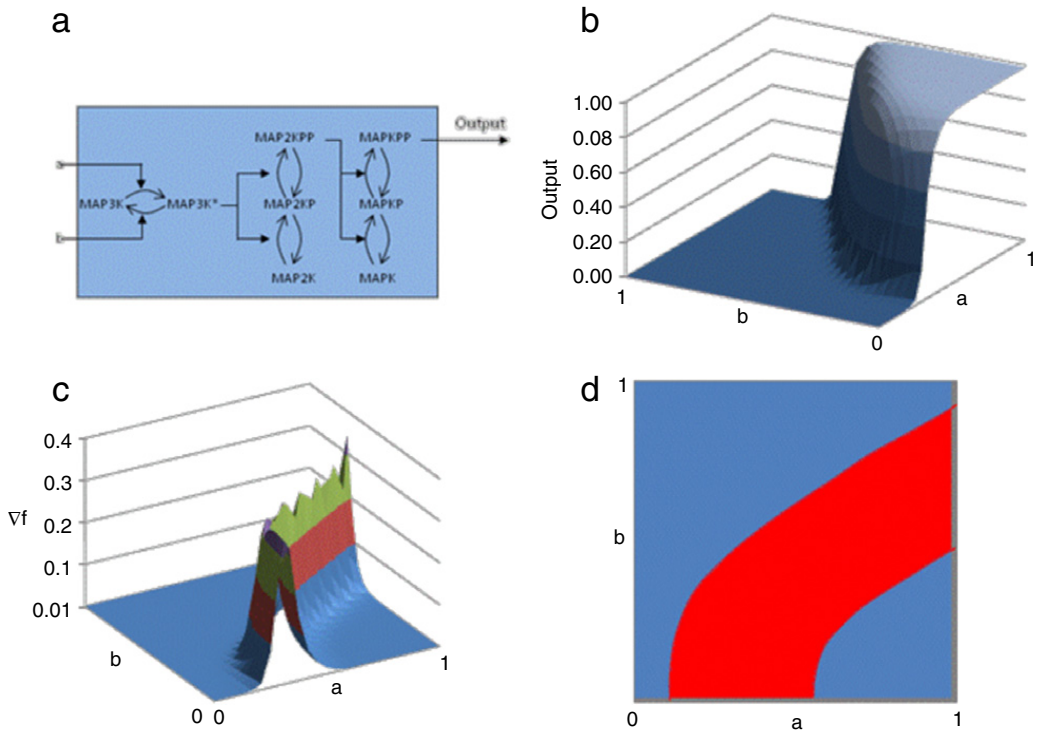

Fig. 6. Mapping of general MAPK enzyme signaling pathway to sensor node logic. (a) Computational model from [18] used as logic circuit to implement state machine in Fig. 4(a). Activator and inhibitor, a and b, provide input values. (b) Behavior of pathway in (a) is diagrammed as a function of normalized input values a and b. (c) Gradient profile of the output function. Raised area indicates region of high sensitivity to input concentration. (d) Updated version of (c) with blue indicating stable areas of operation. Graph axes reflect corresponding abstracted Boolean logic values.

A slight change in concentration of a near the sensitive region indicated in Fig. 6(c) can be amplified and result in an incorrect response. This is not ideal in our application, and this can result in unnecessary invocation of the network layer. The likelihood of such occurrences can be reduced by selecting input concentrations (a, b) such that the gradient values of the output function, are minimized in the vicinity of each point. Where possible we choose operating values so that the region described by $(a \pm 2\sigma_a, b \pm 2\sigma_b)$ has no large gradient fluctuations. This is illustrated in Fig. 6(d), which indicates the regions of stable operation. Using this method, corresponding values for logic 0 and logic 1 are 0 and 0.1 μmol, respectively for a_0 and a_1, and 0.1 μmol and 100 μmol respectively for b_0 and b_1. It should be noted that this technique requires that the extracellular chemical signals that drive the nano sensor are transduced to correct intracellular concentrations of input chemical inside the cell (i.e. $[a]$ and $[b]$).

4.3.2. Network/addressing layer

This section describes an address encoding method for Ca^{2+} signaling. As mentioned earlier, we used Goldbetter et al.'s model for signal-induced Ca^{2+} oscillations from [15]. Control of cytosolic Ca^{2+} is achieved by coupling the output of the enzyme based protocol circuit to the IP3 pathway discussed in Section 4.2. For simplicity, we simulate the coupling of the enzyme logic operation from Fig. 6(a) to IP3 production by making the IP3 component of the model dependent on enzyme logic output o.

Each nano sensor is engineered to transmit unique Ca^{2+} signal which cannot be replicated through the aggregation of other sensor signals in the same network. This can be accomplished by manipulating the sensitivity and type of IP3 receptors in the cell. For example, in [27,39] cells are genetically engineered to express IP3 receptors to affect the encoding of IP3 mediated Ca^{2+}. Therefore, the source address of the signal can be encoded using either the amplitude or frequency of the calcium signal. We simulated both amplitude and frequency modulation of Ca^{2+} by applying inputs to the enzyme logic to alternate state of the nano sensor between "*Send*" and "*Sense*".

Table 1
Address encoding for nano sensor using amplitude of Ca^{2+} wave.

Node	Ca^{2+} amplitude (μM)
1	0.75
2	1.5
3	2

4.3.2.1. Amplitude modulation. The assumption of our proposed solution is that the receiving BioMEMS sink must have the ability to detect varying Ca^{2+} amplitudes for a network of n sensors. The receiving BioMEMS can then translate any received calcium signal into a corresponding address of a node. For example, using the data in Table 1, a Ca^{2+} signal of amplitude 2.25 μM indicates *node* 1 and 2 have detected an event.

We applied inputs to the enzyme logic that triggered a state change from "*Sense*" to "*Send*". By altering the efflux rate of Ca^{2+}, it is possible to tune the upper steady state value for each sensor. We tuned two nano sensor cells, indicated by node 1 and node 2 in Table 1 (and from the scenario of Fig. 6(c)) to exhibit upper Ca^{2+} concentrations

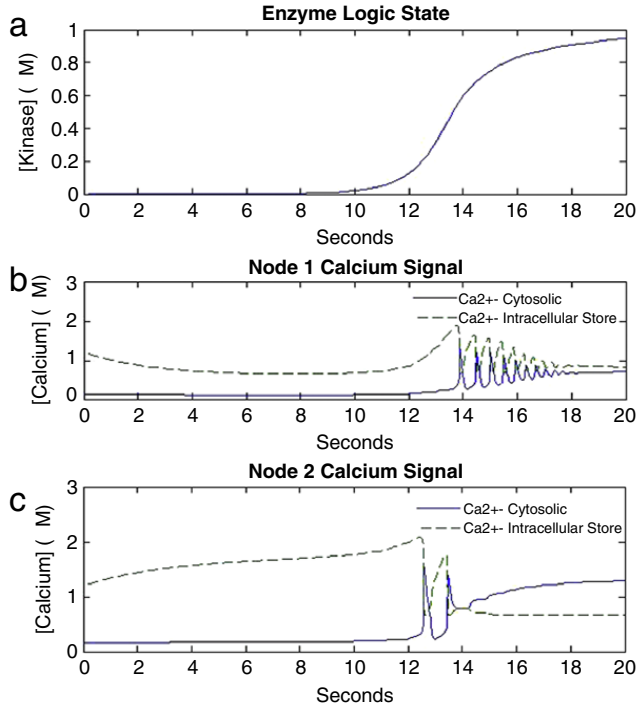

Fig. 7. Amplitude modulation of cytosolic Ca^{2+}. (a) Response of enzyme based circuit to detectable event. This represents transition of biological cell based sensor from send to sense state. (b) Resulting cytosolic calcium concentration in node 1. Cytosolic calcium (solid blue line) transitions from lower stable state to upper stable state. Biosensor cell tuned to upper stable state of 0.72 μM. (c) Node 2 Calcium signal: Upper Ca^{2+} concentration of 1.5 μM.

of 0.75 μM and 1.5 μM respectively. Fig. 7 illustrates the simulation results.

As predicted in [15] we observed cytosolic Ca^{2+} transition from a lower to upper steady state values. Simulations show the desired, stable Ca^{2+} concentrations are maintained in each node. The simulation also shows short burst of Ca^{2+} oscillations occurred during steady state transitions. This occurs when IP3 saturation levels move between 30% and 70% as logic state transitions occur. Since the ultrasensitive response is only exhibited in the feed-forward or activation mechanism, this behavior is particularly apparent going from lower to higher states (i.e. "*Sense*" to "*Send*"). While this does not affect this case study, it would have implications for synchronous or temporal applications that would require implementation of increasingly complicated logic and concatenation of gates where quick state transitions would be required. By selecting optimal input concentration values, the effects of noise are minimized and 95% of input values result in correct response. However, during simulations, some occurrences of erroneous output were observed. This was more likely during $s = (a_0, b_0)$ because of this point's proximity to the area of high sensitivity.

This simulation provides a theoretical solution to network addressing through concentration encoding in nano sensor networks where each sensor's signal can, in theory, be translated to one of a set of mutually orthogonal digital signals. The application of this approach has several practical challenges: (1) there will be physical limitations to the range of Ca^{2+} concentrations possible in an engineered cell; (2) the scalability of this approach is uncertain and the BioMEMS must be able to discriminate between every possible state; (3) the degree of fidelity of this model is unknown in relation to how Ca^{2+} concentration levels can be tuned, and (4) pre-existing knowledge of all nano sensor's location is required.

4.3.2.2. Ca^{2+} *Frequency encoding.* Frequency encoding is also possible by tuning the cell based nano sensor such that the output of the controlling enzyme logic results in sustained calcium oscillation. The frequency of calcium oscillation in the unstable region is dependent on active IP3 levels present in the cytosol and the sensitivity of IP3 receptors integrated to the endoplasmic reticulum. An illustration of the 3 nano sensors from Fig. 4(c) transmitting at different frequencies to a BioMEMS is illustrated in Fig. 8. By tuning each engineered cell to produce a specific frequency, each nano sensor can transmit a calcium wave whose frequency is unique among all other sensors. This can be accomplished by engineering the expression, sensitivity and type of IP3 receptors in the cell, which is similar to solution proposed for amplitude modulation.

Sustained oscillations occur when the IP3 saturation levels is in the region of 30%–70%. Frequency analysis of the generated signals show distinct frequency components with a lower bound of 0.07 Hz and upper bound of 0.2 Hz corresponding to possible frequency range of the model. Similar to the amplitude modulation approach, we assume that the receiving BioMEMS sink is designed to transduce incoming Ca^{2+} signal and filter for sensor frequencies. This could be achieved by convolving incoming signal with detectable frequencies. This approach is effectively a frequency spectrum modulation solution where each receiving BioMEMS is allocated a continuous range of the available frequency spectrum. As each sensor node/disease is associated with a specific sensor, the BioMEMS device should have the capacity to deduce what sensors transmitted the signal and map that to a particular disease indicator.

4.3.3. Transmission

The effective communication distance is governed by the speed of the calcium wave and the decay of the signal as it travels from cell to cell. In [29], Nakano et al. found that experimental results of calcium signaling compared favorably to simulated results using an extended model of calcium signaling developed by Goldbetter et al. [15]. The experiments by Nakano et al. show that cells that form the channels propagate the signal using their own cytosolic calcium, amplifying the signal to increase the propagation distance through calcium-induced calcium release mechanism [25]. The model showed propagation of intercellular Ca^{2+} waves through at least 50 cells with favorable cell–cell coupling parameters.

5. DNA based protocols

In this section we will describe the protocols using DNA based computing. Using DNA based computing leads to a number of advantages that are different from enzyme based computing. Firstly, DNA in its natural form

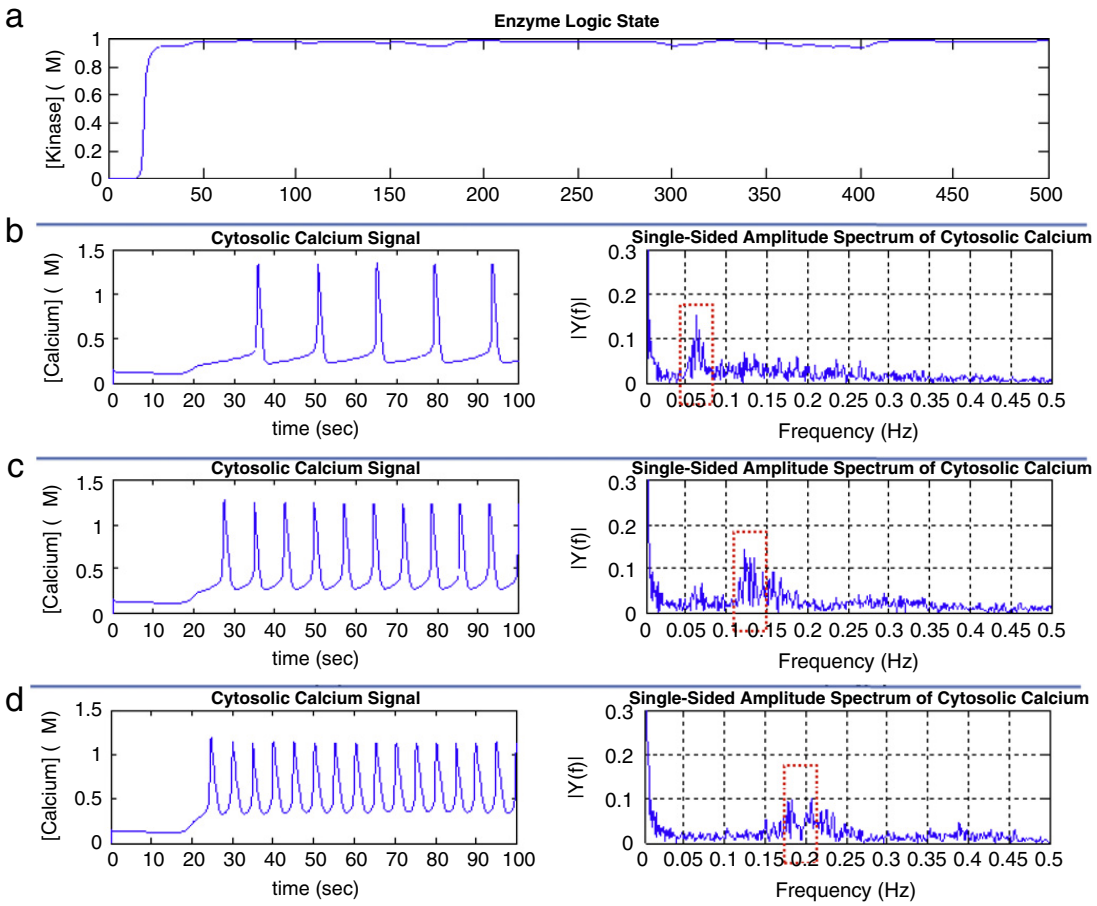

Fig. 8. Frequency modulation of Ca^{2+} signal. (a) Output from enzyme logic computation. (b) Cytoplasm Ca^{2+} oscillations for IP3 saturation of 35% ($c = 0.35$). (c) Cytoplasm Ca^{2+} oscillations for IP3 saturation of ∼50% ($c = 0.5$). (d) Cytoplasm Ca^{2+} oscillations for IP3 saturation of ∼65% ($c = 0.65$). Main frequency components are highlighted in frequency spectrum and correspond to frequency band of each sensor.

has tremendous capabilities of representing information, largely based on arrangements of nucleotide sequences [1]. Secondly, DNAs have capabilities to control a number of other elements and operations within the cell. We exploit these two key properties to develop protocols with higher functionalities that are a challenge for enzyme based solutions. Before we describe our proposed solution, we will first present a background description of DNAs.

5.1. DNA

Every organism has a genome that contains the information required by the cell to construct all the proteins and other molecules that the organism requires to survive. This information is encoded into the sequence of DNA. DNA consists of two long polymers of simple units called nucleotides, where the polymers have a backbone of sugars and phosphates. Attached to the sugars is one of four bases, and the sequence of these bases encodes the information. These DNA molecules are in the nucleus, and produces proteins in the cytoplasm by special molecular complexes called *ribosomes*. A gene produces a protein by creating a copy of itself called *messenger RNA* (*mRNA*). The mRNA travels to the cytoplasm where the ribosome will use this as a template for the production of a protein. A single gene can transcribe many mRNA molecules and a single mRNA is translated into a protein.

Small interfering RNA (siRNA) is another class of RNA that is involved in the RNA interference (RNAi) pathway. The siRNAs are RNA molecules about 20 to 25 nucleotides long with short overhanging ends. RNAi is used to control gene activity and is commonly used in biomedical research to study the function of genes. A method used to produce siRNA is to synthetically modify cells by introducing plasmid based expression vectors into the cell that can express short hairpin RNA (shRNA). The shRNA is then cleaved by a Dicer into active siRNA and we exploit this characteristic in our solution. The following sections give a more detailed description of this process.

5.2. Layered DNA based protocols

The mapping of the proposed DNA based protocol to network protocol is illustrated in Fig. 9. As shown in the figure, this specific solution has closer matching to current TCP/IP based protocol, which in turn increases the functionalities of the protocol stack.

Inspired by Rinaudo et al. in [20], our solution proposes the creation of a synthetic cell based sensor in which the protocol components are encoded by genes made of engineered DNA molecules. In this solution, messages to be transmitted are represented as RNA molecules. The information coding of the RNA is formed at the application layer. However, the creation of the RNA information

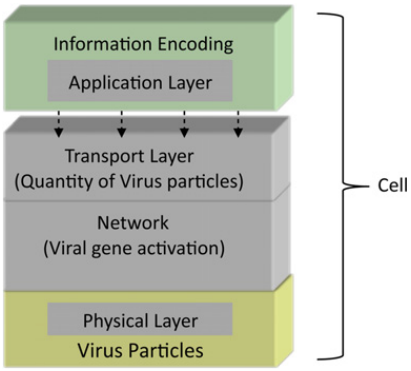

Fig. 9. Proposed DNA based protocols.

molecule is beyond the scope of this paper, therefore, we assume that the RNA messages are already present in the cell. The main carrier of information for this specific solution will be through viral particles. There are a number of advantages in using viral particles, and in our particular case, the advantage is in the addressing functionality. One common application of viral vectors in molecular biology is the transportation and delivering of genetic payloads to biological cells. In particular, lentiviral vector constructs are particularly effective for delivering siRNA expression vectors to biological cells [45]. Also a key characteristic of lentiviral vectors is their ability to target both dividing and non-dividing cells [42].

One of the functionalities of the transport layer in communication protocols is ensuring the reliability of packets transmitted over the networks. In the case of the DNA based protocols, the reliability of message transmission can be controlled through the quantity of virion production. The network layer will then take care of the addressing of the viral envelope, where the virus encapsulating the message represents the physical layer transmission.

5.3. Message packing

Fig. 10 shows the message packing protocol of the DNA based nano sensor. In this subsection we will describe the operation sequence for message packing using the DNA based protocol. The components of the message packing process includes: a viral gene, a short hairpin RNA (shRNA gene), message mRNAs, RNA Induced Silencing Complex (RISC), and Dicer. Both virus particles and shRNA are generated from the viral [19] and shRNA genes [31], respectively. Dicer is used to segment shRNA into small interfering RNAs (siRNAs) [3], while RISC is the silencing machinery that uses siRNA to target and destroy mRNA [12]. The continuously produced shRNA are designed to silence the viral mRNA molecules, where the shRNA is cleaved by the Dicer enzyme to create siRNAs. These siRNAs will then bind to the RISC and separate the two strands of the siRNA. Upon separation one strand will be discarded, while the remaining strand is used to target and destroy viral mRNA (this is from the transcription process of the viral gene). The targeting process is based on complementary base pairs in the siRNA and the target mRNA, and is required to be very accurate. Therefore, the cleavage of the viral mRNA will prevent it being used as a template for viral protein production, thus preventing virus production in the cell. This represents the dormant state of the nano sensor cell, and is shown in Fig. 10(a).

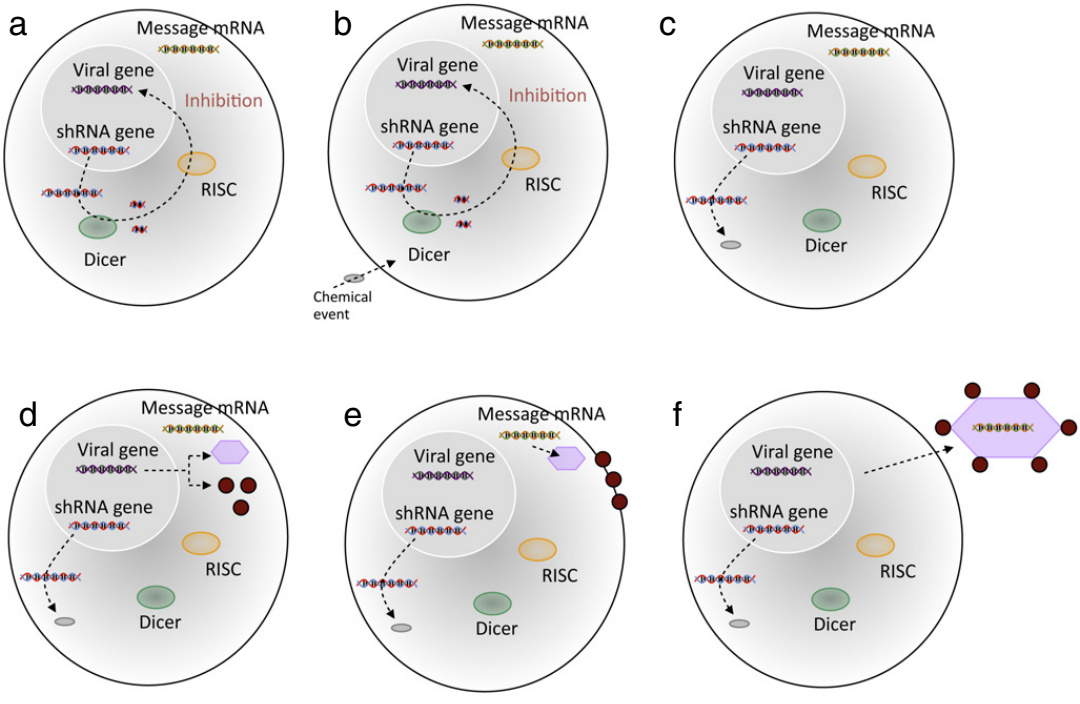

Fig. 10. Sequence of operation for message packing in DNA based protocols.

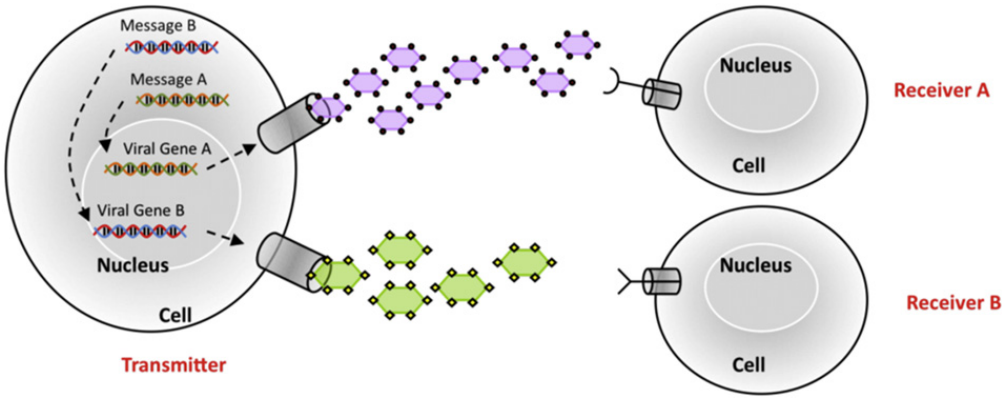

Fig. 11. Addressing using viral based carriers.

The shRNA is also designed to interact to a chemical event such as the occurrence of toxic molecules, and this is shown in Fig. 10(b). In [3], An et al. propose a shRNA based molecule sensor that used an *aptamer* to bind to a detectable molecule. Aptamers are DNA or RNA sequences that bind to specific target molecules, when they are attracted through high affinity. Therefore, an application for toxic molecular detection is to use an aptamer in the loop region of the shRNA, which will bind to any target molecules present in the cell and prevent shRNA cleavage by Dicer (Fig. 10(c)). However, since our proposed solution focuses on the transportation protocols of the messages, we do not delve deep into the shRNA silencing process. A key advantage of using an aptamer based solution is that the aptamer is part of the shRNA molecule and can be produced as a single unit. It would also be possible to produce two different shRNAs where the aptamer of each of the shRNAs can target two different toxic molecules (this would, therefore, increase the potential of the nano sensor for multiple applications). For example, a bio-nano sensor could be engineered to detect and respond in the same way to two different chemical species.

By having the shRNA aptamer region interact with a chemical event, the Dicer action will be suppressed, which in turn will suppress the RISC operation. This will lead to the transcription process of the viral gene to generate viral particles, as shown in Fig. 10(d). The process of creating the viral particle will then absorb the message mRNA, because of specific packaging signal code of the mRNA. The virion generation will create an envelope around the message as it buds out of the cell. Each virion that encapsulates the message has a distinct surface protein that can be attracted to specific receptor of distant cells (receiver nodes). Therefore, through this property, an addressing process can be achieved, which is illustrated in Fig. 11.

5.4. Viral production performance

We will now present results from the simulation of virus production using the Cell Designer [14] simulation tool, which is shown in Fig. 12. We are interested in the effect of RNA silencing mechanism on viral production in a synthetic nano sensor. Our model combines both shRNA and viral production components and assumes that the cell

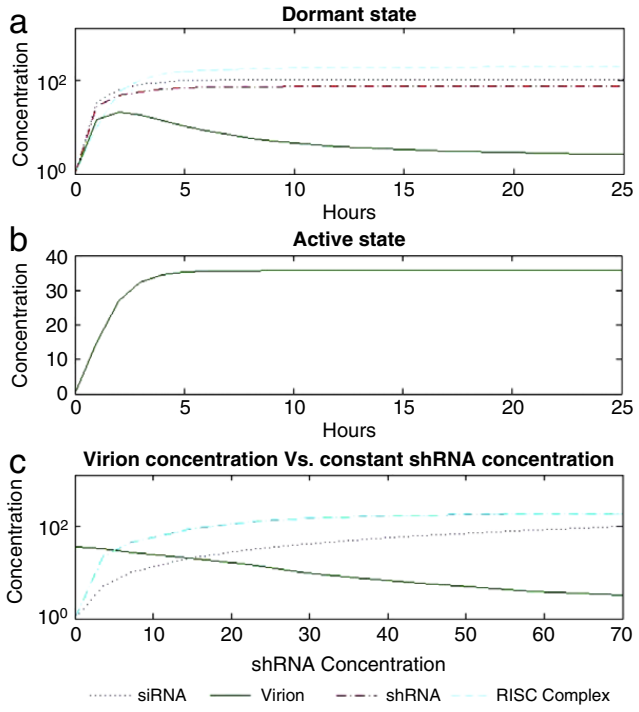

Fig. 12. Dynamics of RNA silencing and virus production. All concentrations units are number of particles per cell.

is engineered to continuously produce shRNA, virions, and message mRNA in parallel.

The viral gene silencing component of the model is based on the RNAi models in [7]. Based on data in [37] for lentivirus, we assume a virion production rate of 36 virions per hour per cell before RNA silencing.

Fig. 12(a) shows the behavior of the cell in its dormant state (i.e. no detectable molecules present). We assume both RISC and Dicer already exist in the cell in sufficient quantities to facilitate their associated reactions. As can be seen, viral production is significantly reduced but not completely silenced through RNA silencing. Fig. 12(b) shows virion production in active state behavior whereby

siRNA generation is suppressed completely. Fig. 12(c) shows the effect of shRNA concentration on siRNA and viral production in the cell. Increasing the concentration of shRNA results in a corresponding increase in siRNA and siRNA–RISC complexes.

These properties observed through our simulation work can help in the design of the transport level reliability and application layer requirements. For example, in our solution, a cell's ability to communicate reliably with a receiving node is governed by secretion rate of transportation particles (i.e. virion). Therefore, the occurrence of a "critical" concentration of a detectable substance should produce sufficient quantity of virions to reach its intended target within a particular time period. The properties observed in this simulation could be combined with diffusion based intercellular communication analysis [13] to calculate effective and maximum communication distances for viral based communication networks.

6. Discussion

In this section we will present a comparison discussion of the two proposed solutions. As presented earlier, both approaches for molecular scale protocol developments have a unique mechanism that supports their operation as well as deployment environment. In the case of enzyme based computing, the logic operations are performed in the cytosol. There are a number of advantages for this, which includes the speed of operation as well as simplicity. In the case of speed of operation, this is largely due to the operation of the signaling pathways in the cytosol. The simplicity stems from the low requirements needed to engineer the cell to support these protocol developments. As discussed earlier, through varying detection of multiple pathway operations, logic circuits can be extracted to control the messaging of Ca^{2+} signaling. Besides the short speed required to perform the protocol operation, the other advantage is the speed of signaling using Ca^{2+}. In our case study presented in the paper, the application of this approach is for direct and short distance messaging to a BioMEMS sink. However, there are also a number of challenges with the enzyme based protocol. Firstly, only limited information can be transmitted. In the event that richer information is required, this will add more complexity to the logic circuit. Since each logic gate corresponds to a pathway, higher number of logic gates will map to higher number of pathways. Invoking multiple pathway signaling can lead to higher interference within the cytosol, making the judgments of the output much more difficult. Naturally occurring biological mechanisms have intracellular spatial compartments that can increase specificity and fidelity in chemical reactions. In [4], we described a method to support parallel enzyme computation using protein scaffolds whereby components involved in computation are closely assembled into cascades to increase specificity and reduce unwanted interactions with other chemicals. Such technique could be applied to support more complex logic circuits for more specialized protocol functions. At the same time, the routing process for the enzyme based computing approach will be very challenging. An element will be required in the intermediate node to evaluate the incoming Ca^{2+} and understand the frequency and amplitude of the signal, before the same signal can be replicated for the next node.

The DNA based protocol on the other hand has the main advantage of ensuring richer information encoding through manipulation and organization of mRNA nucleotides. The main application of this approach would be for devices that will require rich sensing capabilities. The protocol is most ideal for periodic monitoring and sensing, or long-term treatment. These applications are suitable for the slow information transmission of this proposed solution, where the time to activate a decent quantity of viral particles can take very long. This is largely due to nature of the virus movement, which is based on pure diffusion. The main challenge of this approach, however, is in the complexity of the protocols. This complexity rises from the high coordination required for each of the steps of the protocol and the ability for the virus to grab the message molecule before leaving the cell. In order to enable the multiple steps and coordination, cells will require new internal architecture organization. One approach to enable this could be through spatial compartmentalization [21], which uses artificial compartments engineered within the cell. Another challenge is the amount of destination nodes that each node can transmit. This limitation is largely due to the limited number of viral genes that can be inserted into the nucleus (since each viral gene corresponds to a specific destination).

7. Conclusion

The evolutionary changes of communication systems have witnessed new emerging systems of various scales and sizes, with different capabilities and functionalities. A very good example of this was the emergence of sensor networks a few years ago that enabled communication between miniature devices. Through these evolutionary phases, the design paradigm of communication protocols has been consistent, with minor changes on specific layers of the protocol. Molecular communication is part of this evolutionary change, and aims to enable communication between nano devices in biological environment. In this paper, we propose solutions for protocol development for molecular communications using molecular computing. The solution includes enzyme based computing that supports short distance calcium signaling and DNA based computing that supports long distance communication using viral particles. The two protocol solutions are designed as a platform for nano device communication with different applications and communication distances. This paper has also presented a discussion on the two approaches, including the advantages as well as challenges that remain for future work.

Acknowledgements

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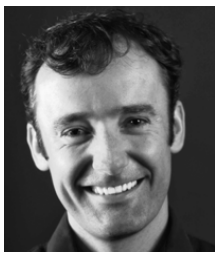

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A.5 Artificial Backbone Neuronal Network for Nano Scale Sensors

In our work so far we have concentrated predominantly on the mechanisms and techniques to create protocol stacks for molecular communication nanonetworks. As highlighted in section 1.1.4, one of the main challenges in protocol development for molecular communication nanonetworks is incorporating the diverse range of molecular communication techniques available at the physical layer.

This paper investigates the use of neuron cells to create an artificial backbone neuronal network to be used by nano scale sensors. We explore how neuron cell characteristics affect the performance of an artificial neuronal network through the simulation of several network topologies.

Artificial Backbone Neuronal Network for Nano Scale Sensors

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***Abstract*—Communication between biological based nano scale devices is a crucial component of future applications in nanotechnology. This paper explores the creation of a backbone communication network for nano scale sensors using neurons. We investigate how neuron cell characteristics affect the performance of neuronal based network and highlight several key characteristics compared to conventional wire based networks. Finally, we investigate four network topologies through simulation.**

I. INTRODUCTION

In recent years Nanotechnology has received tremendous attention due to its potential application in various medical fields. The area of nano communication has recently been introduced to enable communication between nano scale devices [1]. This new capability can increase capabilities and functionalities of nano devices, in particular from perspective of their application base. As the popularity of this research area increases, a number of different solutions have been proposed. Currently, two approaches have been investigated, which includes electromagnetic wireless nano networks [8] and Molecular Communications [9] [10]. Electromagnetic based nano sensor devices resemble conventional sensor networks, and uses similar design concepts and communication capabilities. On the other hand, molecular communication is a new paradigm shift from conventional communication devices, where communication is performed on underlying biological environment. In such cases, the information is usually transformed into bio-molecules at the physical layer [6], before being propagated to the receiver. A number of different approaches have been investigated, and one potential approach is through the use of neurons.

In this paper, we aim to show how neurons can, within their intrinsic properties, form a network, to be used, for molecular communications. Neurons are specific type of cells that form highly interconnected networks, where the signaling is performed through dedicated cellular synapses (Fig. 1). The scenario we concentrated in is a fixed wireline backbone network that supports communication between distributed nano scale sensor and a sink. Given the biological nature, the physical shape and characteristics of neurons, our aim is to associate with neurons the molecular communication function of being the interconnecting links. Due to the capabilities of neurons to forms complex connections (e.g., web), we believe that this could be used to form wireline infrastructure for communication. In particular, our aim is to develop the backbone infrastructure at the basis of simple information transfer. Thus, this paper will evaluate how different geometric topologies can be used as neuronal backbone networks, and how each of these shape's influence can have diverse blocking probabilities. Therefore, the simulation work, here presented, is developed to show the associated performance for each topological shapes taken into examination.

The paper is organized as follows: Section II will present the related work, while section III will present background information on neurons and their characteristics. Section IV will describe our backbone neuronal network, while section V will present the simulation work. Lastly, section VI will be the conclusion.

II. RELATED WORK

The related work section is sub-divided into two sections, which includes current state of the art in molecular communication, and neuronal networks.

A. Molecular Communications

As described earlier, one form of communication for nano devices is through molecular communication [9], [10].

Molecular communication can be sub-divided into two propagation approaches, which are passive and active transport. Passive transport is usually associated to diffusion based propagation of molecules [11] [12]. However, in the case of passive transport, there is not an identified unidirectional path in the propagation of signals. For instance, one example is the natural occurring calcium signalling that propagates between the connexons of cells or by larger static magnetic fields [7]. Whereas an example of active transport includes the use of microtubules to connect between cells, where the molecular motors are used to transport information [13]. Another approach that has been proposed is through the use of nano scale cargoes which can be loaded onto kinesin-coated surface, that act as cargo transporters [9]. Through the use of labeling process of using single-stranded DNAs, the cargo can then selectively unload in specific locations.

The approach that we propose in this paper is availing of the active transport approach although using a different physical layer component: a neuron. While current approaches can allow directionality in transporting molecules, we believe that the process of forming interconnecting networks, similarly to conventional networks, will be difficult by using the above described approaches. Conversely, we believe that through neurons we can achieve closer characteristics to conventional communication networks.

B. Neuronal Networks

Kotsavasiloglu et al. [2] [3], studied the signaling performance of neuronal networks under varying condition. The aim of the study was to investigate the resilience of neuronal networks when synapses fail, where such failure can be due to aging or diseases. The authors performed simulations on the neuronal network of healthy neurons, and varied the synapse failure rate, refractory periods, excitation synapse ratio, as well as synapse delay. Here we show that neuronal networks are very resilient and are able to maintain high level of activity up to 70 – 80% destruction of synapses. Forming a pre-defined geometry and connectivity of neuronal networks has been investigated in a number of works. Breskin et al. [5], showed that connectivity of neural networks is based on Gaussian distribution rather than scale free network. Gabay et al. [4], developed a new approach of pre-defined geometry of neuronal network clusters using carbon nanotube clusters. In the proposed approach, neurons migrate on low affinity substrate to high affinity substrate on a lithographically defined carbon nanotube template. Once neurons have reached their destinations, they send neurites to form interconnected networks. This approach improves on previous methods, where neurons interconnecting the networks collapse during migration.

While a number of works have investigated neuron networks from a networking perspective, our approach taken is different. In our proposed approach presented in this paper, we aim to show from natural occurring physiological perspective.

III. NEURONS

A neuron is the basic unit of a neural network (node) and has the ability to process information packages in the form of electrical and chemical signals. The classical structure of a neuron consists of 4 specific regions including the cell body, dendrites, the axon and the axon terminal [16]. The cell body or soma contains organelle for protein synthesis while branching from it are dendritic extensions which receive incoming chemical signals from abundant neurons simultaneously. The axon transmits incoming electrical impulses or action potentials to the nerve terminal where it forms synaptic contacts with other neurons [17]. Hence, the action potential depolarizes the pre-synaptic membrane opening voltage operated channels (VOCs) and potentiates the influx of extracellular calcium [12]. Increasing intracellular calcium concentrations initiates exocytosis of synaptic vesicles containing neurotransmitters. The axon terminal is the area where a synapse occurs between the pre-synaptic neuron and the post-synaptic neuron and it is within this synaptic cleft that information of the signal is relayed via excitatory or inhibitory neurotransmitters. In this perspective, the travelling information package can be considered the action potential generated by a cascade of chemicals events that take place on the surface of the cell membrane.

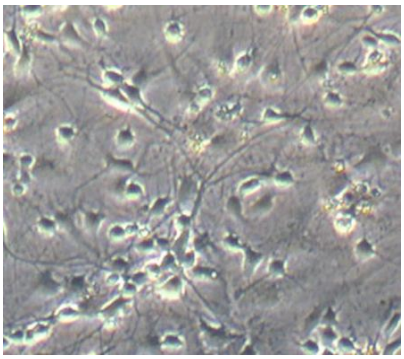

Figure 1. Examples of pattern of connections in a self organised network of neurons; please note cell bodies (or soma), axons (larger filaments) and dendrites (smaller filaments). (magnification x20).

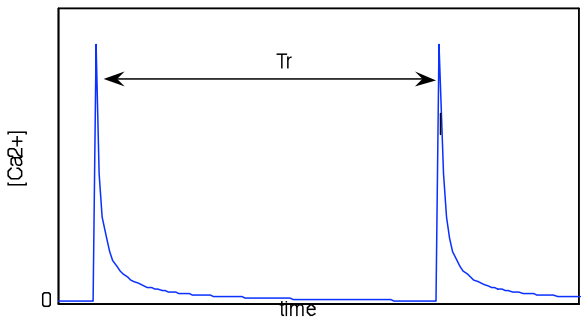

Figure 2. Intracellular Ca^{2+} concentration in a neuron. Ca^{2+} release events must be separated by at least the refractory time T_r, the time required to replenish internal Ca^{2+} stores.

In other words, the action potential can be seen as a travelling gradient of ions concentration (Na^+/K^+, Ca^{2+}) along the whole length of the cell structure. Tracking the movements of these ions may lead to a new way to interpreter the synaptic communication and dynamics within a neural network.

There are a number of inherent differences between neuron link and a wireline communication link. First of foremost, conventional communication links have specific bandwidths that are able to support multiple traffic flows. This is not the case for neurons, which are only able to transport a single flow at any one time. Secondly, once a flow is terminated within a conventional communication link, the link becomes empty and is ready again to accept a few traffic flows. However in the case of neurons, there is a refractory period, as shown in Fig. 2 which prevents the neuron-link to be used for a defined period of time. Similarities lies on the signal distribution. As signals are propagated from neuron to neuron, this could be compared to a burst-traffic behavior found in conventional communication links. At the same time, delays in intermediate nodes of a communication network (due to queuing delays) are very similar to synaptic delays found between the junctions of the neurons.

IV. Backbone Neuronal Network

As described earlier, our aim is to create an active molecular communication transport network using neurons. Mazzatenta et al. [15], showed that electrical signals delivered via single-wall carbon nanotubes can directly activate neuronal signaling. This approach could provide a physical interface mechanism for nano scale sensors and neurons. In our work, we design the neuron networks for specific applications and these can be illustrated in Fig. 3, where we have a number of sensors that are connected via a network of neurons to transmit information to a receiving sink (targeted-neuron for communication actuation). Therefore, the key issue here is the ability to maximize the coverage and enable collection of information from majority of sensors to send information to the receiver sink. Therefore, in order to ensure, the geometric shape of the backbone is crucial. Fig. 4 illustrates the three types of topologies that we are considering for our investigation, which consists of a simple Bus (Fig. 4 (a)), Star topology (Fig. 4 (b)), Spiral shaped topology (Fig. 4(c)), and a Tree shaped topology (Fig. 4 (d)).

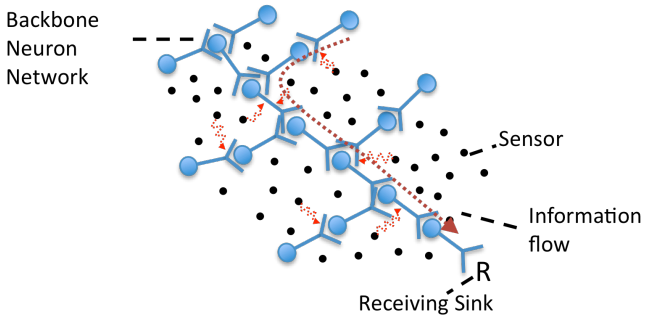

Figure 3. Sensor connected through Neuron Network

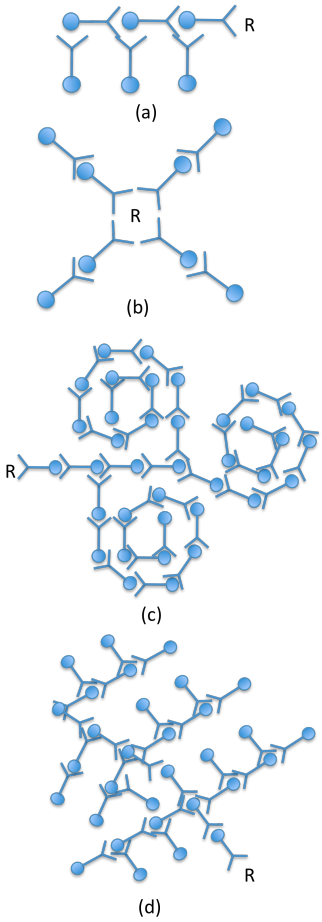

Figure 4. Topologies for Neuron Network Backbone (a) simple Bus topology, (b) Star topology, (c) Spiral shape, (d) Tree topology

For the latter two topologies, our aim is to build on the bus topology structure to develop other types of topologies that can maximize the information coverage. On this, one crucial characteristic that will influence the performance of each topology is the timing processes within a neurons. This timing issue ranges from the timing for the action-potential to induce the electrical signalling to the refractory period of Ca^{2+} as well as associated delay of signaling in the synapses. Therefore, by taking these into account our work provides an opportunity to allow multiple devices to transmit on the same bus link, provided the delays of transmission are properly triggered. For example in Fig. 5, four neurons are connected to a single receiver *R*. This example shows how multiple neurons within the bus can fire without leading to signaling interference. If neuron *A* first fires, neuron *D* will be able fire no later that d_{Delay} to minimize interference at *D*. This is provided that sum of d_{Delay}, the signal propagation of D ($t_{p,D}$) and the refractory time of D ($T_{r,D}$) is less than the sum of propagation time of A ($t_{p,A}$), B ($t_{p,B}$) and C ($t_{p,C}$).

An example illustration of Ca^{2+} disruption caused by two neurons firing close to each other is illustrated in Fig. 6. In this illustration, 16 neurons are connected in a bus topology configuration. Fig. 6 (a) illustrates when two neurons are fired, without any collision events, where the Ca^{2+} in each neuron are fired sequentially. This is when neuron 1 fires at t=0, and this is

followed by neuron 3 firing at t=15. There is no collision leading to no disruption in Ca^{2+} signalling since the firing of neuron 1 occurs way before d_{Delay}, which allows the neurons, down the bus line, to recover from the refractory process; this allow for both transmissions to successfully propagate. On the other hand, Fig. 6 (b) illustrates when two neurons are closely firing to each other and thus leading to collisions which disrupts the firing of Ca^{2+}. As we can see from the figure, the firing of Ca^{2+} in each neuron is terminated after neuron 6 due to the collision (please note that the Ca^{2+} is not the signal that propagates between the cells, but the signal used to invoke the neurotransmitters).

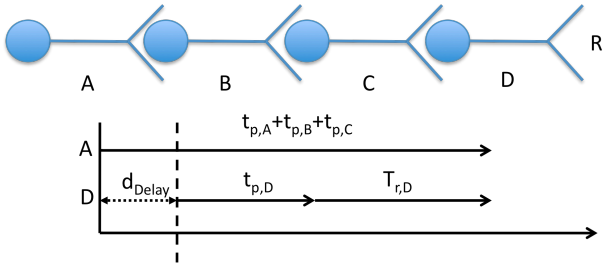

Figure 5. Propagation timing between neurons

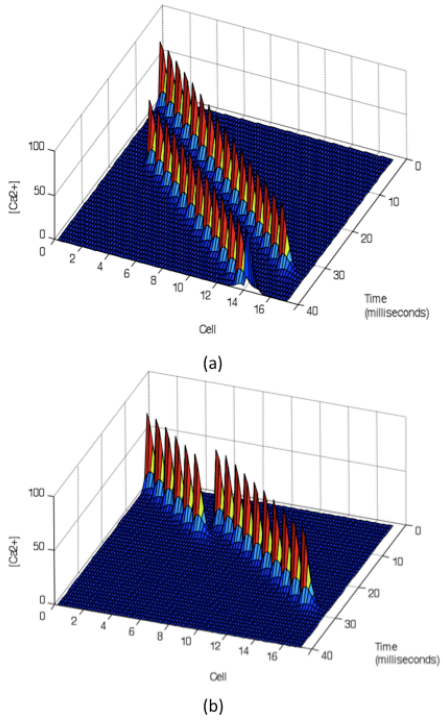

Figure 6. (a) Ca^{2+} signalling in straight bus topology for firing from neuron 1 and 3, (b) Ca^{2+} disruption caused by collision caused by close firing from neuron 1 and 7

V. SIMULATION

This section will present the simulation work conducted on the different topological shapes shown in Fig. 4.

A. Single Bus topology results

The first set of simulation is based on evaluating the performance of the bus topology as we vary the number of devices on the bus and the transmission rate. The parameters for the simulation environment are shown in Table I. The transmission events are performed for $\tau = 0.005$ s, in agreement with the neuron physiology.

TABLE I. SIMULATION PARAMETERS

Simulation Time	5 s
Transmission window	4.5 s
Device Transmission rate	0.1 – 2 ms

Fig. 7 and 8 shows the performance of the bus topology with varying transmission rates and number of devices.

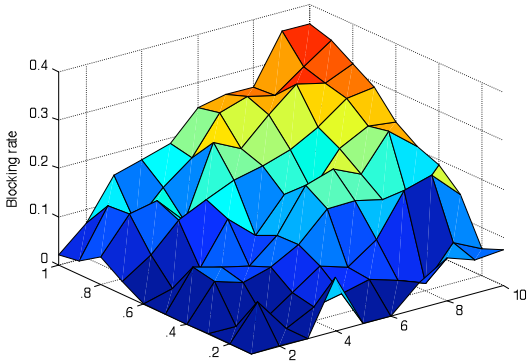

Figure 7. Performance of bus topology for varying number of devices and transmissions per second (0.1 – 1)

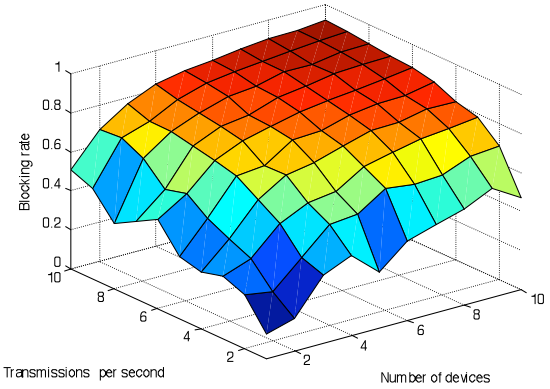

Figure 8. Performance of bus topology for varying number of devices and transmissions per second (1 – 10)

In the case of Fig. 7, the transmission rate is between 0 – 1/ s, while in Fig. 8, this rate change between 2 – 10/s; and this is

in line with what we expected when increasing the number of transmission, when the number of collisions increases. There is, however, very little difference between the highest number of devices and low number of devices, when the transmission rate is low. We believe this may be caused by the close proximity of devices which led to a larger number of collisions. Obviously in the case of Fig. 8, with very high transmission rates correspond a very high rate of collision.

B. Comparisons between Bus, Star, and Spiral topology

In this sub-section we present the result of comparison between the Bus, Spiral, Tree and Star topology. The parameters used in the simulation are presented in Table II. Configurations for the listed topologies are as follows: the number of devices was fixed to 10 devices, while the number of transmissions events varied. The Bus topology contains 13 neurons connected in a line, with the sensors connected in fixed locations of [1, 2, 3,4, 5, 6, 7, 8, 9, 10], while the Receiver is located at neuron 13. The Spiral topology is based on Fig. 4 (c), where three spirals are connected to a single bus line. Each spiral has 13 neurons connected where the location of sensors connected to the neurons for each spirals are located at Spiral-1: [1, 5, 8, 10], Spiral-2: [1, 3, 9], and Spiral-3: [2, 6, 9]. Furthermore, the tree topologies have six short branches connected to a shared media with nodes distributed across the branches as illustrated in Fig. 4(d). In the case of the Star topology, this consists of 3 buses connected to a receiver at the centre. Even in this case, each bus line has a length of 13 neurons.

TABLE II. SIMULATION PARAMETERS

Simulation Time	0 to 1000 * τ (10 sec)
Transmission window	0 to 900 * τ (9 sec)
Probability of device transmitting at time $n\tau$	$1x10^{-3}$ - $1x10^{-2}$
Device Transmission rate	0.1 – 1/second

Similarly to previously reported simulation, the calcium model used in our work is based on Fire-Diffuse-Fire model [14]. In order to make the simulation more efficient, it is assumed that the transmissions (transmission event) only take place at time $n\tau$, integer multiples of the calcium release time constant τ. For our simulation, we took $\tau = 0.01$s. Interestingly, the blocking rate between the Bus and Spiral are very close, as shown in Fig. 9, since the Spiral topology is essentially a bus topology with a Donut shape. The tree topology shows a marginal improvement compared to Bus and Spiral due to the increased length of the communication network. This increases the average distance (in nodes) between transmission events and, therefore reduces the likelyhood of blocking the transmission during the initial stages of the simulation. Furthermore, the Star topology has a lower blocking probability. This is mainly due to the fact that the star topology does not have a single shared media like the bus, which minimizes collissions during the transmission.

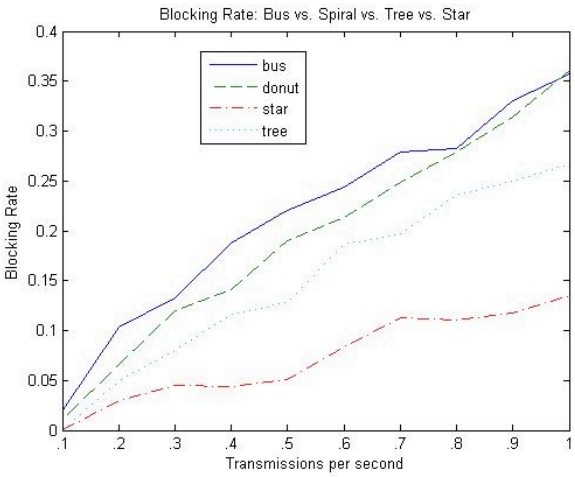

Figure 9. Comparison between Bus, Star, and Spiral topology

VI. CONCLUSION

Molecular communication is one form of communication between nano- machines (devices, organisms), and therefore represents a new paradigm shift from conventional communication systems. In this paper, we have proposed the use of neurons to form interconnected networks for the active transport of defined action-potential events in a molecular communication model. The aim of our proposed approach is to create a communication network from biological components. We believe that this could be best achieved through neurons that are able to form web of interconnection similar to conventional wireline networks. Four topologies of neuron interconnection were modelled and their performances of blocking rate under varying number of devices and transmission were evaluated. This allowed us to determine if the patterns of transmitted events can be influenced by the topological shapes with an increased success rate in transmission.

While this work is the first step towards enabling the creation of artificial neuronal networks, we believe that this could create a new form of active transport of events across molecular networks. Our future work will focus at creating more refined communication network design based on physiologically relevant neuronal networks to address, or resolve, specific signalling demand.

ACKNOWLEDGEMENTS

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A.6 Development of artificial neuronal networks for molecular communication

In this paper we extend the previous workshop paper in appendix A.5 and address the challenges identified therein. The paper also contains experimental results, performed in collaboration with CRANN institute at Trinity College Dublin, that demonstrate signalling in neuronal networks. Also, we use a novel genetic algorithm approach to overcome network collisions in neuronal networks.

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Development of artificial neuronal networks for molecular communication

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ABSTRACT

Communication at the nanoscale can enhance capabilities for nanodevices, and at the same time open new opportunities for numerous healthcare applications. One approach toward enabling communication between nanodevices is through molecular communications. While a number of solutions have been proposed for molecular communication (e.g. calcium signaling, molecular motors, bacteria communication), in this paper, we propose the use of neuronal networks for molecular communication network. In particular, we provide two design aspects of neuron networks, which includes, (i) the design of an interface between nanodevice and neurons that can initiate signaling, and (ii) the design of transmission scheduling to ensure that signals initiated by multiple devices will successfully reach the receiver with minimum interference. The solution for (i) is developed through wet lab experiments, while the solution for (ii) is developed through genetic algorithm optimization technique, and is validated through simulations.

1. Introduction

The field of nano/molecular communication is a new area of communication research paradigm, aiming to provide communication capabilities between nanoscale devices [2,23]. Increasing the communication capabilities of nanoscale devices can increase their capabilities and application base, in particular, in the healthcare and pharmaceutical industry. The current research of communication at nano and molecular scale include both molecular communication as well as electromagnetic based nanoscale communication [2,1]. Molecular communication enables communication to be performed between nanoscale devices by utilizing biomolecules as a communication medium, while electromagnetic based nano communication allows communication between nanodevices using wireless technology.

In this paper, we will focus on molecular communication, particularly investigating the use of neurons as a networking component. We will discuss a number of development aspects of neurons that can be implemented as an underlying network to support molecular communication, which includes the following (i) the ability to artificially invoke and suppress signaling in neurons, and (ii) a scheduling design in a neuron topology that could minimize signaling interference. In the case of (i), the solution can be used to allow external devices to interface to neurons and switch the neurons to signal transmission.

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Once devices have switched and signaled the neuron, then the case of (ii) can be used to ensure the signaling transmitted through the neuron network will minimize interference to ensure that signals propagated will reach the destination. We discuss a number of characteristics of neuronal transmission as signaling of Ca^{2+} ions trying to highlight the strict relation between these ions and the transmission of the action potential from a pre-synaptic neuron to the post-synaptic neuron. The signaling behavior of the neurons will be considered in the design process for the scheduling protocol for the neuron networks. Our approach used for designing the scheduling algorithm is based on optimization techniques. Optimization is a common approach used in various network design problems, such access scheduling [19,18], routing and resource management [14] as examples.

The objective of our paper is to present design solutions that could enable nano/molecular communication researchers to use neurons as a communication network component, to transfer and re-use common design approaches, and apply best practices found in conventional communication network to nanoscale communication networks. The paper is organized as follows: Section 2 presents the related work on molecular communication and neuronal networks. Section 3 presents background information on Neurons. Section 4 presents the design of neuron to nanomachine interface, while Section 5 presents the design of the scheduling transmission over the neuronal network. Lastly, Section 6 presents the conclusion.

2. Related works

The related work is separated into two sections, which includes molecular communication as well as neuron networks.

2.1. Molecular communications

A number of solutions have been proposed for molecular communication in recent years. Example of these solutions includes the use of propagation based on molecular diffusion (e.g. calcium signaling [25]), walkway based molecular propagation [10,9], or bacteria networks [5]). Current research activities are investigating the mathematical theory of molecular communication channels, highlighting the challenges of molecular communication based nanonetworks with much addressing the physical mechanisms of molecular communication and molecular communication based nanonetworks.

A key challenge in molecular communication research is noise characterization in volatile aqueous molecular communication channels. For example, in [28,29], Pierobon and Akyildiz presented physical and stochastic noise analysis models for diffusion based molecular communication in nanonetworks. The authors develop a mathematical expression for physical processes underlying noise sources while their stochastic approach characterizes noise sources as random processes. Another challenge in molecular communication is data encoding. Typically, two mechanisms are proposed, which includes concentration encoding and molecular particle encoding. In [21], Mahfuz et al. explore solutions to concentration encoding in diffusion based molecular communication systems. The authors explores both sample and energy based decoding schemes whereby the former samples at a single instant and the latter accumulates samples over a defined period.

Accurate computational and energy models are also a key aspect in the development and understanding of communicating nanodevice. While many energy management models exist for larger scale networks, they are generally not applicable to nanonetworks where nanodevices would be more inaccessible and expected to be more energy self-sufficient. In [16] Kuran et al. propose an energy model for molecular communication via diffusion. Work is also being conducted at the data link and network layers in nanonetworks. In [24], Nakano et al. present a model for in-sequence molecule delivery inspired by out-of-order delivery techniques in computer networks. Simulations using several molecular propagation mechanisms reveal motor driven random walks result in higher probability of in-order reception. As expected, increased symbol transmission periods and receiver buffering time significantly increase probability of successful in-order reception.

While numerous works have investigated communication network theory for diffusion based molecular communications, the area of active transport for molecular communication is still in its infancy. In particular, the investigation into the use of neuron networks for active transport, which is what we aim to investigate in this paper.

2.2. Neuron networks

Neurons form highly complex network, in which they are responsible for processing information in the brain. Kotsavasiloglu et al. [12,15] developed computational models to study the behavior of biological neural networks and also discussed the connection between computational and biological models. The authors performed simulations on the neuronal network of healthy neurons, and varied the synapse failure rate, refractory periods, excitation synapse ratio, as well as synapse delay. Firstly, they focus on the signal transmission and analysis, and investigated the existing critical crossover value regarding the loss of connections by studying the robustness and degradation of dynamics on a network by varying the number of connections which corresponds to the synapses of the biological neural networks. The authors later developed a model to discover the results of synapse loss which can occur in biological systems under certain diseases, such as Alzheimer's and Parkinson's [15].

Breskin et al. [3] set up an experimental design to determine statistical properties of living neural network. They separate the initially connected network to the fully disconnected smaller clusters and use a graph-theoretic approach to study the connectivity. It is observed that if the network's connectivity increases, a percolation transition occurs at a critical synaptic strength. Their study also indicates that connectivity of neural networks is based on Gaussian distribution rather than scale free network. Gabay et al. [6] developed a new approach of pre-defined geometry of neuronal network clusters

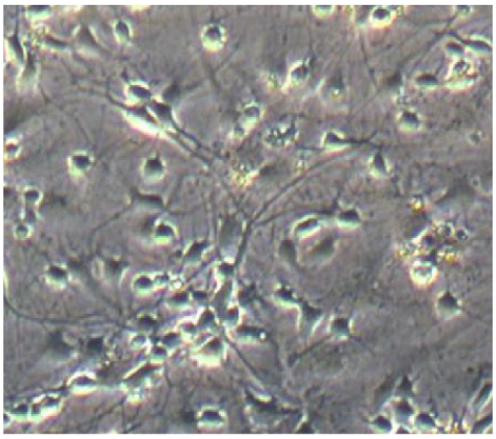

Fig. 1. Examples of pattern of connections in a self-organized network of neurons; please note cell bodies (or soma), axons (larger filaments) and dendrites (smaller filaments). (magnification ×20).

using carbon nanotube clusters. In the proposed approach, neurons migrate on low affinity substrate to high affinity substrate on a lithographically defined carbon nanotube template. Upon reaching the high affinity substrates, the neurons will form interconnected networks by sending neurite messages. A number of works have also looked at mechanism to stimulate neurons, such as the use of LED matrix [8].

Numerous works have studied network properties of neurons, such as connectivity and topology formation network. However, we take a number of these studies further by utilizing the understanding of these networks, and the ability to use them to support molecular communications.

3. Properties of neuron signaling

This section will describe the properties of neurons, where these properties will be used for the design process described in the later sections. Neurons are a basic unit of a neuronal network, where its structure is composed of the cell body, dendrites, the axon and its terminals [4]. Neurons have tremendous abilities to self-organize and form networks through transmission of neurites, as discussed earlier in the works of Gabay et al. [6]. Fig. 1 shows an example of neurons that have self-organized into a network.

As a component of the neural network, neurons are able to process information in two forms, which are electrical and chemical signals. The signaling process is created from an action potential depolarization in the pre-synaptic membrane that opens the Voltage Operated Channels (VOCs), which in turn potentiates the influx of extracellular calcium ions (Ca^{2+}) [31]. Therefore, increases in intracellular calcium concentration initiate exocytosis of synaptic vesicles containing neurotransmitters. The neurotransmitters are transmitted through the synapse between the axon terminal of the pre-synaptic neuron and the post-synaptic neuron. Therefore, the action potential can be seen as a traveling gradient of ions concentration ($Na^+/K^+, Ca^{2+}$) along the whole length of the cell structure. Based on this property, the information that is transferred from one neuron to the next can be considered as the action potential that is generated by a cascade

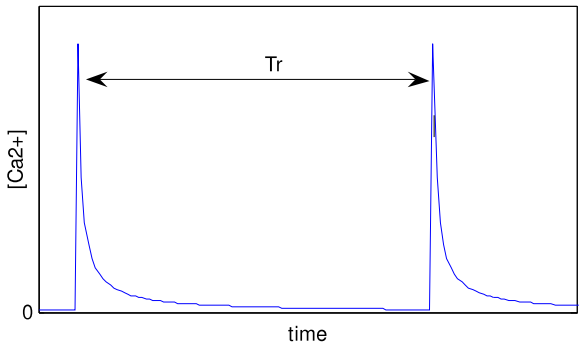

Fig. 2. Intracellular Ca^{2+} concentration in a neuron. Ca^{2+} release events must be separated by at least the refractory time T_r, the time required to replenish internal Ca^{2+} stores.

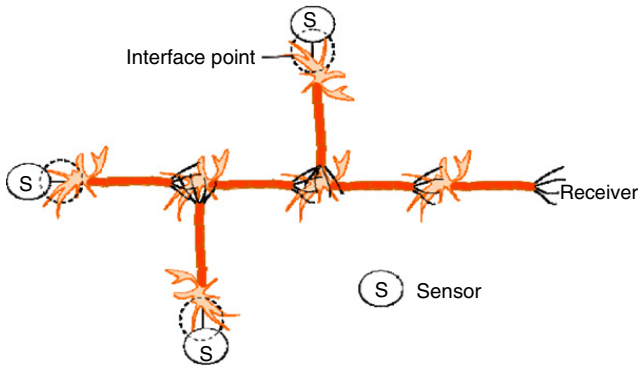

Fig. 3. Interface point between sensor devices to neuron.

of chemical events occurring on the surface of the cell membrane.

Calcium signaling has an inherent property, which is illustrated in Fig. 2. Once calcium within a neuron is activated, there is a refractory period known as T_r. During this refractory period, the neuron will not be able to process any other incoming signals from other neurons, until the T_r period is complete.

4. Design of neuron activation interface

In this section we will present the design of interface to activate neuron signaling. Our scenario application is illustrated in Fig. 3. In our scenario we have sensors that are interfaced to neurons, and activates signaling, where the signaling is propagated to the receiver. Therefore, a requirement is the sensor to be able to emit an agent that can activate the signaling. It is most ideal if this requirement could be achieved through a non-invasive approach (e.g. the firing of the neuron can be controlled externally). Our main objective is to invoke trans-membrane calcium chemical signaling which in turn will induce signaling between the neurons. Therefore, our aim is to also model the calcium signaling that is artificially induced, and to measure this at two different neurons to demonstrate how signals have traveled through the network, as it induces the calcium signaling of the neurons along the path.

We performed experiments to demonstrate this activation process, using primary cortical neuronal cultures obtained from 1-day old rats and plated on customized

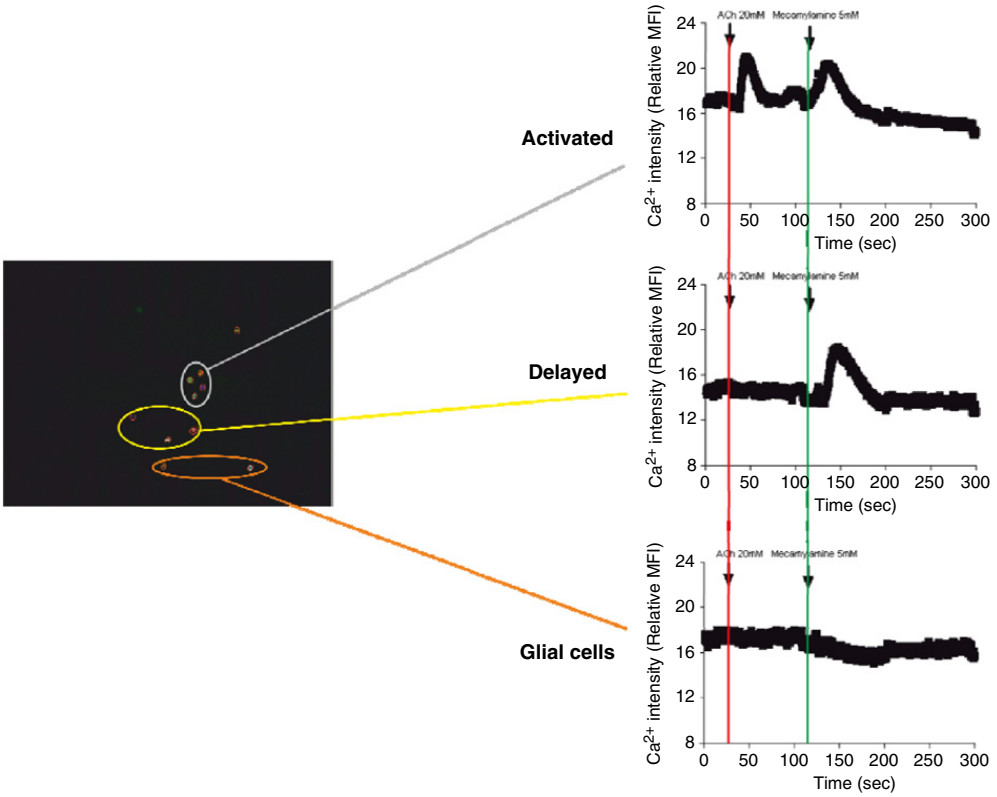

Fig. 4. Fluorescence image of neuronal cells recorded over an interval of 300 s. In this experiment the microinjection and diffusive gradient of Acetylcholine (within the first 30 s of recording) and respective injection and inhibition of Mecamylamine (120 s) is illustrated. Plot of Ca^{2+} flow over the 300 s recording showing different response times of clustered neurons according to their relative position. The red vertical line represents the time flag at which the ACh was microinjected, while the green line represents injection of Mecamylamine as inhibitor. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Microelectrode Arrays (MEAs). In this experiment, Acetylcholine (ACh) is the agonist used to stimulate firing of neuronal action potentials while Mecamylamine is the antagonist which suppresses neuronal firing, thus exhibiting a switch-like function. Neural communication can be demonstrated by Ca^{2+} signaling using *in vitro* cultures. Increasing intracellular Ca^{2+} signifies neuronal activation by enhancing neurotransmitter release and thus potentiating action potentials between neurons. Fig. 4 demonstrates the results from the experiment to show the activation of neurons. Relative mean fluorescent intensity as a measure of basal Ca^{2+} activity was recorded. For application of ACh (20 mM) at 40 s, a steady increase of Ca^{2+} ions was detected while addition of Mecamylamine (5 mM) indicated that Ca^{2+} ion flow was suppressed since fluorescence was reduced below basal levels. Therefore, demonstrating the ability for external sensor devices to use these agents to switch on/off signaling onto the neuron network. At the same time, the experiment also strengthens the idea that Ca^{2+} is a valid marker to track signals transmitted between two neurons.

Fig. 5 demonstrates the results of the experiments on the MEA, where measurements are taken at different points of the network. As we can see in Fig. 5(a), the majority of neurons were in a dormant state during basal measurement. However, following ACh application (Fig. 5(b)), potentiates neuronal firing, thus increasing Ca^{2+} fluorescence intensity. Conversely, Mecamylamine (Fig. 5(c)) suppresses neuronal firing and decreases Ca^{2+} intensity. In this particular experimental example, the white arrow is where the ACh is applied, and shows the neuron firing, and another measurement point is taken at the black arrow, showing the signal propagation. The application of ACh could represent a digital 1 bit transmission through the neuronal network.

5. Design of scheduling protocol for neuronal network

The previous section presented our solution for initiating Ca^{2+} signaling on a neuron from an external sensor device. However, if the sensors emit ACh randomly to initiate signaling, this could lead to large number of interferences in the neuron network, which in turn can lead to corruption of information in the receiver. Therefore, a next requirement in our design is a scheduling protocol to ensure that minimum interference will be encountered during transmission to ensure that signals received are not corrupted. We return back to our scenario presented in the previous section, which is illustrated in Fig. 6. As illustrated in the figure, our aim is to ensure that initiated signals will not result in any collisions during the transmission along the network to a single receiver.

The main aspect of this study is the interaction between the normal activity of the neuron network and the packages of information "injected" simultaneously on the very same "line". As discussed above, the neurons possess a refractory period in which no signals can be transmitted. This results in a sort of bus timing for the signals to pass

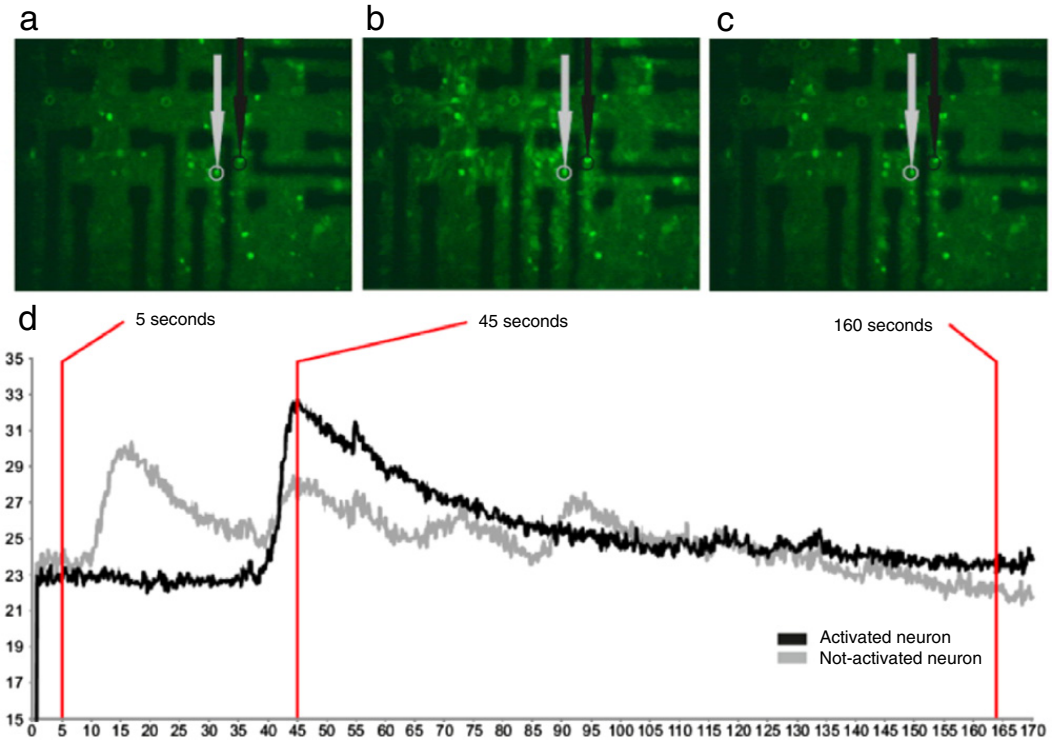

Fig. 5. Fluorescent intensities of intracellular Ca^{2+} in primary cortical neurons cultured on customized microelectrode arrays (MEAs) stained with Fluo-4 AM. The red vertical lines represent the overall course of Ca^{2+} fluorescent intensities for the sample of neurons at the three distinct time points of the experiments. (a) Measurement of Ca^{2+} fluorescent intensities in two sample neurons (gray and black circles) at baseline after 5 s; (b) Fluorescent intensity in the two neurons at 45 s following ACh (20 mM) application demonstrates an increase in Ca^{2+} ion flow as indicated by a brighter intensity of the cell body in the black circle; (c) Fluorescent intensity of the two sample neurons following Mecamylamine (5 mM) addition at 160 s from beginning of recording. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

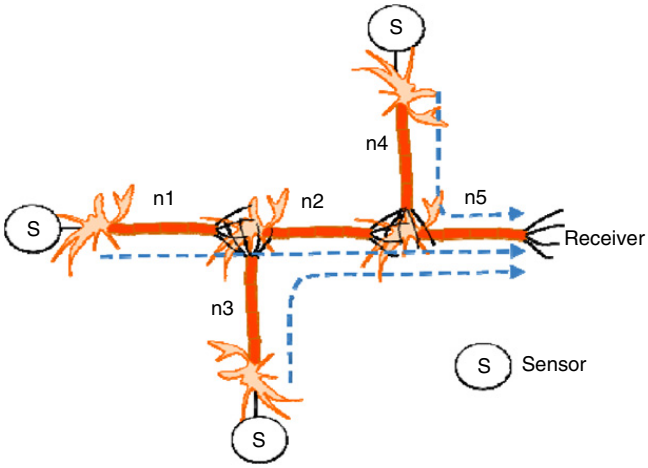

Fig. 6. Sensor transmission along neuronal networks to single receiver.

through the network. Matching the electric signal carried by the action potential with the Ca^{2+} it may be possible to create a parallel communication system that will not compete with the natural one. Fig. 7 illustrates our single bit—Time Division Multiplex Access (TDMA) scheduling, where we aim to schedule the firing of specific neuron with respect to time. Fig. 7(a)–(d) shows the neurons that are fired with respect to time, while Fig. 7(e) shows this from a time division perspective (each color represents a single bit of information transmitted from a specific sensor). Fig. 7(e) also shows the single bit transmission for each time slot. The reason that only a single bit is transmitted per slot is based on two assumptions—(1) there are only two amplitudes that can be produced for bit 1 and 0, and (2) after transmitted, the neuron has a waiting time of T_R during the refractory period, where this waiting time can be used by another sensor to transmit to maximize parallel transmission.

Before we explain our TDMA scheduling algorithm, we will first describe some inherent differences between a neuron link and a wireline communication link. In most communication networks, each link will usually have different bandwidth values. Therefore, the routing process between a source to destination will usually be able to

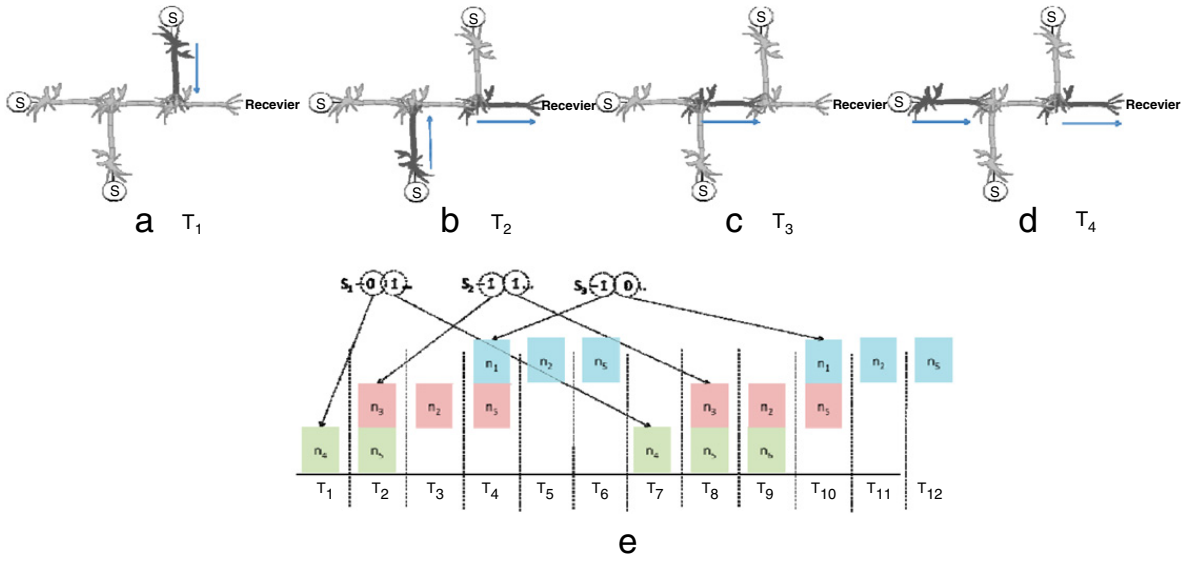

Fig. 7. Single bit—TDMA scheduled transmission from different sensors along a neuron network (each color represents a single bit information from a sensor). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

accommodate a number of flows. However, this is different in the case of a neuron link, where each link of the neuron can only accommodate finite number capacity (this capacity may only represent a single bit). At the very same time, once a neuron is fired, as described in Section 3 (Fig. 2), there is a refractory period where the Ca^{2+} returns to the intracellular stores. During this refractory period, no signal can be transmitted through the neuron. However, this is different from a conventional wireline link, where flows that are terminated can accommodate new flows immediately. While there are differences, there are also similarities between the two. Firstly, as signals are propagated from neuron to neuron, this could be compared to a burst-like traffic behavior found in conventional communication links. Secondly, delays in intermediate nodes of a communication network (due to queuing delays) are very similar to synaptic delays found between the junctions of the neurons. We will consider a number of these properties when we are designing our TDMA scheduler for the neuronal network.

The scheduling design for the neuron network is based on an optimization problem, and the specific technique that we have applied is based on genetic algorithm. The following sections will describe background information on genetic algorithm and some of their applications, the problem formulation for the TDMA scheduling protocol, and we will also present the simulation results of our proposed design algorithm.

5.1. Genetic algorithm

Genetic Algorithm is an optimization search heuristic [7]. The search process is through a guided search that is inspired from the natural evolution. The first step is by creating a random initial population of solutions. This initial population will then go through a series of evolutionary generations, where an optimum solution will slowly emerge based on certain genetic operators. These operators include crossover, mutation, and selection. Each solution of the population is called a chromosome, and has an associated fitness function. Therefore, the optimal solution will be achieved, once the fitness function of the population starts to converge and stabilize.

Genetic algorithm has been used in a number of different communication network problems. Example of these problems includes communication network routing [30,13], as well as network services [32,17,26]. In these various applications, genetic algorithms have produced improved performance compared to numerous approaches, both in design and run-time applications. Therefore, in the same way that genetic algorithm has been successfully applied to communication network problems, we aim to re-use this approach for design of scheduling in neuronal networks. At the same time, since our problem is defined as an optimization problem, we believe that genetic algorithm is an appropriate approach.

5.2. Problem formulation

The objective of our design problem is to maximize the number of signaling messages (x_{si}) as well as minimize the time difference between the sensors that release the ACh ($t_{a,si}$) to activate the signaling, over a period of time T_p. Information that is provided for the optimization problem includes the number of sensors $s_i = (s_1, s_2, \ldots, s_i, \ldots, s_M)$, where M is the total number of sensors; location of the sensors as to which neuron this is connected to; total number of N neurons, where $n_j = (n_1, n_2, \ldots, n_j, \ldots, n_N)$; as well as the neuron topology. Therefore, the objectives can be represented as:

$$maximize \sum_{i}^{M} \sum_{y}^{T_p} \sum_{j}^{N} x_{s_i,j,t} \tag{1}$$

$$\sum_{k.l}^{s_i} \frac{1}{|t_{a,k} - t_{a,l}|}, \quad l \neq k, \tag{2}$$

subject to:

$$n_{i \neq j,t} \quad (i,j) \in N \tag{3}$$

$$t_{a,j} \leq T_p \quad j \in s_i, \tag{4}$$

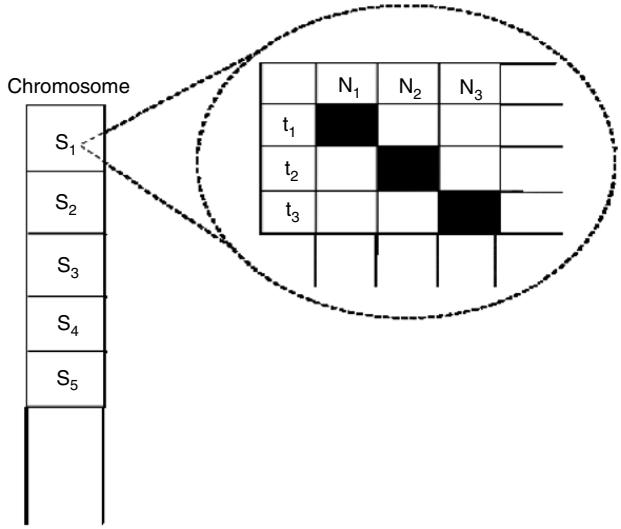

Fig. 8. Chromosome structure which is composed of an array of sensor, which contains a two-dimensional array composed of time steps and neurons in the topology.

where x is the message passing through a neuron. Objective (1) is to maximize the total number of parallel number of neurons transmitting messages in the topology, where $x_{s_i,j,t}$ is the message from sensor s_i passing through neuron j at time t. Objective (2) is to minimize the difference in time ($t_{a,si}$) that sensor s_i fires the neuron through the release of ACh (the aim here is to pack the firing time between the sensor to be as close as possible). Eq. (3) specifies that at a specific time t, the neurons that are fired in the topology must be unique, while Eq. (4) specifies that all initial timing of a sensor t_a must be less that the T_p.

5.3. Genetic operators

Chromosome: As described earlier, the genetic algorithm operates by evolving over a set of solutions, until an optimum solution is reached. Each solution in a genetic algorithm is referred to as a chromosome. For our specific application, the chromosome structure for our solution is illustrated in Fig. 8. The chromosome is composed of a set of sensors, where each sensor is composed of a two-dimensional array, where the rows represent the time steps for the whole time period T_p, while the columns represent the neurons of the topology. During the initial population creation, a random initial time t_a and neuron n_a is selected and set to 1. The next period is set to $t_a + t$ and neighbor neuron n_j of n_a, and this continues until we reach the last neuron of the topology or T_p. This procedure is repeated for all sensors. The time steps and neurons that have been set are recorded, so that when there is a conflict, the solution is eliminated, as this is an infeasible solution. The fitness function of each chromosome is calculated as:

$$f_c = \log\left[\alpha \sum_{k,l}^{s_i} \frac{1}{t_{a,k} - t_{a,l}} + (1-\alpha) \sum_{j}^{N} n\right], \quad k \neq l,\ k, l \in s_i. \tag{5}$$

Selection: A roulette wheel selection process is used for selection of chromosome solution for the next generation.

Table 1
Genetic algorithm configuration.

Population size	200
Number of generations	200
Crossover probability	70%
Mutation probability	5%

Table 2
Configuration for Topology 1.

Number of neurons	43
Number of sensors	11
Total time steps	40
Weight (alpha)	0.5

The roulette wheel selection operates as follows: A total sum of fitness f_T for all chromosome is calculated, after which a probability P_S is calculated per chromosome by the ratio of f_c/f_T. Therefore, this ensures that the fitter chromosomes are selected for the next generation.

Crossover: The crossover probability P_{CO} is randomly assigned to each chromosome. After selection of each generation, each chromosome's P_{CO} is checked and compared to a crossover threshold. If the value is over the threshold, a crossover is performed with another chromosome with a higher value threshold. The crossover performed is a single point crossover, where the crossover point is selected randomly at a specific gene in the chromosome.

Mutation: The mutation is performed by checking if the assigned mutation probability P_M is over a threshold. A chromosome selected for mutation is performed by selecting a random time and neuron point in the two-dimensional array of the gene and changing the bit. This is then checked to make sure that it is a feasible solution.

5.4. Performance

We evaluated our algorithm on two neuronal network topology shown in Fig. 9.

A crucial requirement in our performance evaluation is the development of a suitable topology. A number of studies have investigated neuron network topologies. A common topology to represent tree topology of neuron networks is through using *Diffusion Limited Aggregation* (*DLA*) [20]. Through the branching structure, information are transferred and received. We developed a similar random tree-like topology that mimics a dendritic tree of interneuron [20], where we produced two topologies of size 43 neurons and 151 neurons. We evenly distributed sensors in the topology at a ratio of 1/4 to the number neurons. For each topology we only have one single receiver (denoted as R) in the figures.

The configuration parameters for the Genetic Algorithm are shown in Table 1. The number of neurons, sensors, as well as total time steps for the simulation is presented in Tables 2 and 3 for Topology 1 and 2, respectively.

5.5. Genetic algorithm performance

The performance of our fitness function and its convergence speed is shown in Fig. 10. We can see that the convergence to the fittest solution converges much faster for

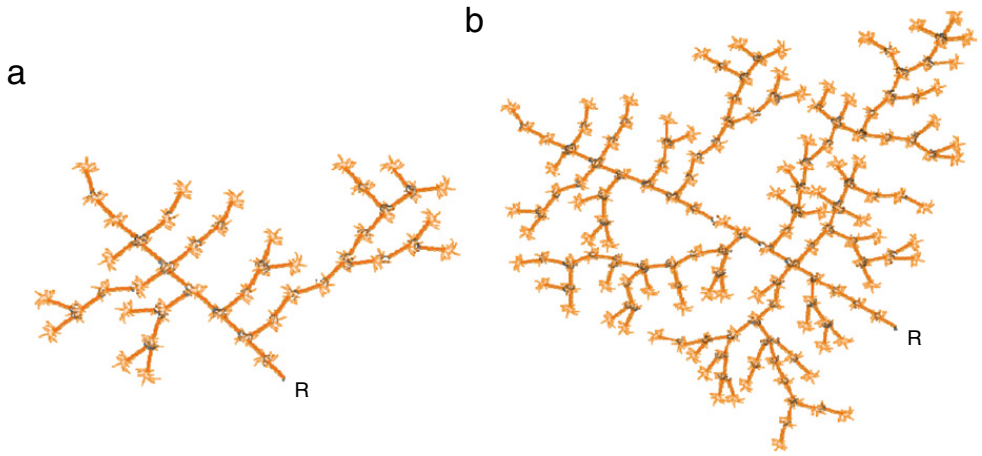

Fig. 9. Topology of neuron network evaluated (a) Topology 1, 43 neurons, (b) Topology 2, 153 neurons.

Table 3
Configuration for Topology 2.

Number of neurons	151
Number of sensors	40
Total time steps	320
Weight (alpha)	0.5

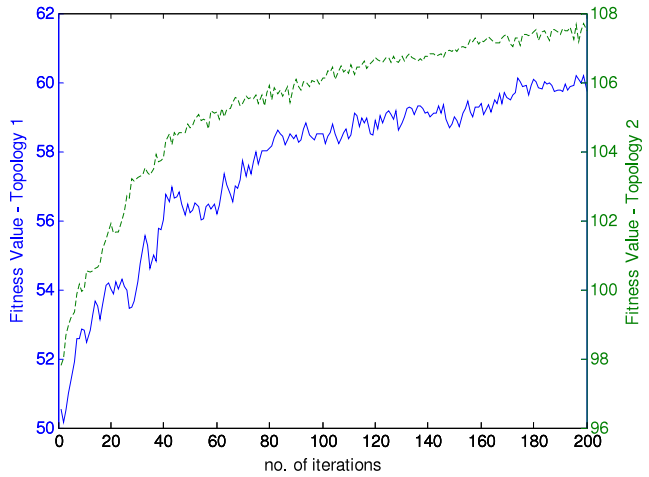

Fig. 10. Convergence performance for Topology 1 and 2 ($\alpha = 0.5$).

Topology 1, compared to Topology 2. For simplicity, we have set the weighting value α of the fitness function to be 0.5. In our future work, we intend to determine the optimal weighting value α.

5.6. Neuron network performance

Simulation results for GA based scheduling designs for both topologies are illustrated in Table 4, where the tests includes the transmission blocking rate, average neuron utilization, average transmission delay. As expected, the GA based scheduling resulted in successful reception of all transmitted messages for both topologies.

Figs. 11 and 12 shows the number of active neurons with respect to the time for the GA based solution and compares this to the random signaling of sensors. The result is aimed to show the number of parallel neurons that are fired in one instance of time. As stated previously,

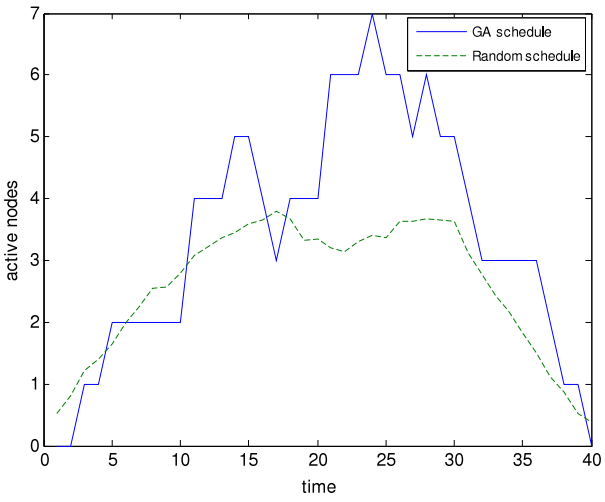

Fig. 11. Comparison of active neurons for Topology 1 between GA and Random ($\alpha = 0.5$).

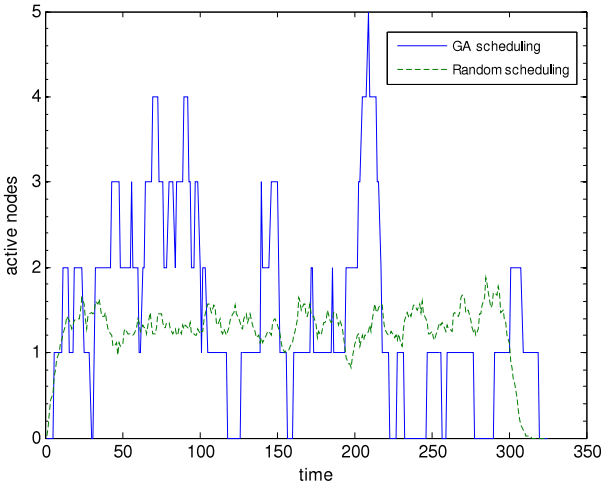

Fig. 12. Comparison of active neurons for Topology 2 between GA and Random ($\alpha = 0.5$).

the goal of the GA fitness function is to maximize neuron utilization and minimize the signaling time between the sensors. For Topology 2, the GA simulation has an average link usage of 1.45 with a minimum of 0 to maximum of 5

Table 4
Simulation results from TDMA scheduling design.

	Blocking rate		Average neuron utilization		Transmission delay (time)		Max. link usage	
	GA	Random	GA	Random	GA	Random	GA	Random
Topology 1	0	0.241	1.36	1.67	5.364	4.711	7	8
Topology 2	0	0.11	1.45	1.24	10.58	9.50	5	6

whereas the random simulation resulted in an average link usage of 1.24 nodes over all simulations with minimum of 0 and maximum of 6. As can be seen in Figs. 11 and 12, the GA simulation exhibits typical characteristics of TDMA scheduling in that the state of the system is fully determinable at any time.

The sensor locations and resulting transmission schedule from the GA is simulated. For random simulation, sensor locations are distributed normally across the neuron network and all transmit events are also normally distributed in the total transmission period (see Table 4). As with GA configuration, each sensor transmits once in the transmission period. We can see that the blocking rate for the random is approximately 0.241 and 0.11 for topology 1 and 2 respectively. The blocking rate is higher in topology one because the transmission events are confined to a much smaller time period and node group. However the average transmission delay is slightly higher than the GA solution. This is expected, since the random signaling does not consider the interference between sensor signaling, and may initiate signaling very close to each other.

The ability to design and construct neuronal networks to specific topology is crucial to the solution that is discussed in this paper. In [11] Jang et al. present a novel method that uses carbon nanotube patterned substrates to direct neuron growth. The authors report highly directional neurite growth along carbon nanotube lines which is attributed to high absorption of neuron adhesion protein by carbon nanotube patterns. This method could be used in our solution to create the neuronal network topologies discussed in this paper.

Similarly, a method to connect bionano sensors to neural networks is essential for our solution. Recent studies have shown that carbon nanofibers can be used to interface between bionano devices and neuron cells. For example, in [27], Nguyen-Vu et al. demonstrated the use of vertically aligned carbon nanofibers as an interface to neuronal networks. The authors predict this technology will have applications in implantable neural devices and the development of neuromodulation based systems. In the context of our solution, it can provide the mechanism by which bionano sensors can interface and communicate via neuronal networks.

6. Conclusion and remarks

As the field of nanotechnology gains momentum through their numerous application base, in particular for healthcare, research in communication capabilities between the devices is still in its infancy. Molecular communication aims to address communication between nanodevices in biological environment. In this paper, we present a development of artificial neuron networks for molecular communications. Inherently, neurons form self-organizing networks that enables information processing. Due to this property, our aim is to design solution that can enable communication between devices connected through a neuronal network. Our scenario is a number of sensors that can transmit information through the neuronal network to a single receiver. Our very first design is to address a mechanism that interfaces between nanodevices to neurons that can initiate neuron signaling. We present our solution through experimental work, where we allow signaling to be initiated through administering Acetylcholine to cultured neuron, and this signaling can be suppressed by administering Mecamylamine. This in turn provides capabilities for nanodevices to create switches as they are interfaced to the neurons. The second stage of our design is to determine optimal scheduled timing of release of Acetylcholine to initiate signaling, in order to minimize any interference in the neuron topology. This is set as an optimization problem, where our aim is to minimize the timing of signaling between the sensors, while maximizing the number of parallel neurons fired. Simulation results have validated our design and comparisons have been made to random signaling of sensors.

The aim of our proposed solution, as described earlier, is to develop molecular communication solutions that can exploit neuronal networks, and at the same time, to design these processes by re-using principles and approaches from communication networks. We believe, that is the first step toward investigating neuronal network as a solution for molecular communication, and can open numerous opportunities for future work.

Acknowledgments

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Appendix. Experimental setup

Primary cultured cortical neurons and plating

Primary cortical neurons were dissociated and prepared from 1-day old Ham–Wistar rats (BioResources Unit, Trinity College, Dublin 2, Ireland) as described by [22]. The

cortices were dissected after humane death and decapitation and meninges gently peeled from neonate brains. Tissue was digested with trypsin from bovine pancreas (Sigma) in sterile PBS and incubated for 25 min at 37 °C. This step was neutralized with trypsin inhibitor type II S: soybean (0.2 μg/ml Sigma) and DNase (0.2 mg/ml). Cells were gently titrated and passed through a sterile mesh cell strainer (40 uM) for single cell suspension. Following centrifugation, cells were re-suspended in pre-heated neurobasal media supplemented with glutamax (2 mM), heat-inactivated horse serum, penicillin & streptomycin (100 units/ml) and B27-supplement.

Cells were seeded onto customized microelectrode arrays (MEAs), fabricated by standard lithographical processes onto borosilicate glass, at a density of 1×10^6 cells/ml coated with laminin (0.05 mg/ml) and incubated in a humidified atmosphere 5% CO_2: 95% air at 37 °C. A sealed gasket made of polydimethylsiloxane (PDMS, Dow Corning, USA) was placed over the cells to contain the neurobasal media to prevent evaporation and housed in a sterile Petri dish.

Calcium signaling

Fluo-4 AM Calcium indicator (Invitrogen, USA) was used as a fluorescent indicator of mitochondrial calcium. The co-culture of neurons and astrocytes on day-in-vitro (DIV) 5–7, were loaded with 4 μM Fluo-4 AM and pluronic F-127 which was dissolved in recording buffer and incubated in the dark for 45 min at 22 °C. Cells were washed and incubated for 30 min at 22 °C. Relative mean fluorescent intensity was measured using optical microscopy.

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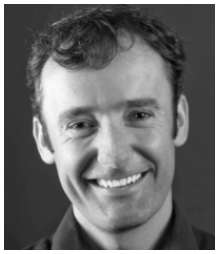

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A.7 Reliability of Multi-path Virus Nanonetworks

The paper in appendix A.6[60] showed how a virus-based molecular communication physical layer can be controlled by an RNAi-based application layer protocol component. In this work, we identified several advantages of using viruses for molecular communication which is discussed in section 2.1.

This paper focuses on the characteristics of the virus-based physical layer and develops an analytic model for calculating reliability in multi-hop virus-based nanonetworks. This can be used to inform protocol development and topology design for virus-based molecular communication.

Reliability of Multi-path Virus Nanonetworks

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***Abstract*—Molecular Communication is an emerging communication paradigm which uses biological particles to encode and transport information. In this paper we present a mathematical analysis of a molecular communication based nanonetwork which uses virus particles as information carriers. The analysis examines the reliability of a multi-path virus based nanonetwork and the effects of physiochemical properties such as diffusion coefficient and particle decay. Based on our analysis, our objective is to create nanonetworks of biological devices within a tissue to support future medical applications.**

I. Introduction

The use of nanomachines within the body has significant potential to create novel health care therapies and treatments. A key ability of nanomachines is to monitor and affect functions at a molecular and cellular level *in vivo*. This ability is supported by concurrent advances in both silicon and biological based nanoscale computation which provides the possibility to create complex nanomachines with enhanced capabilities. For example, existing biological cells can be modified to include genetic based computational components, essentially re-purposing them as nanomachines that can perform a specific tasks such as detecting specific chemical species and actuating a corresponding response. However a key requirement of such applications is the ability to communicate with both peer devices and upstream conventional networks such as body area networks(BANs). This can be achieved though a nanoscale network or *nanonetwork*[1] that can interconnect biological based nanomachines.

There are significant challenges in the creation of biological nanoscale networks[2]: Communication in a biological medium can be unreliable and exhibits significant communication delay relative to conventional data networks. Also, the nanonetwork topology has a significant effect on several important factors such as communication reliability and data throughput. If the nanonetwork is to support critical services then an acceptable level of robustness and redundancy must be present in the network to ensure that the network continues to perform in an acceptable manner, even when parts of the network are not functioning correctly. These issues are not dissimilar to those faced in conventional sensor networks. However, nanonetworks must also take into account domain specific factors such as increased transmission delays, particle decay, and the computational constraints of nanodevices.

In this paper, we analyse the use of lentivirus particles as the carrier for Ribonucleic acid (RNA) encoded molecules in nanonetworks. The combination of viruses and RNA molecules have several interesting characteristics in the context of nanonetworks. One particular benefit is the potential to encode large amounts of information in nucleic acid molecules. For example, recent advances in DNA data encoding[4] make DNA molecules feasible for use as a biological Protocol Data Unit(PDU) at the physical layer.

Lentiviruses provide the following functions in relation to RNA molecules: encapsulation of RNA payload molecules at a source cell; transportation of RNA through a biological medium; insertion of an RNA payload into target cells. These functions are used by viral vectors in gene therapy applications and we propose to use these in the creation of virus based nanonetworks. However, virus particles decay over time and thus can only propagate a certain distance as illustrated in fig. 1. Also, viruses are broadcast in all directions and suffer concentration dilution as the distance is increased from the source. Therefore, similar to wireless communication sources, virus sources will have and effective range beyond which it is unlikely that a successful reception event can occur. By creating a multi-path network topology, the range and reliability of virus based nanonetworks can be improved, whereby intermediary nodes in the network can relay the RNA payload molecule. A particular functionality that we intend to investigate is a redundant multi-path topology approach in order to achieve an acceptable level of network reliability. We analyse two simple multi-path topologies and examine how reliability is impacted for different environment parameters.

The paper is organised as follows: Section II describes related work in this domain which has inspired this research. Section III presents an analytical model for virus multi-path networks. Section IV presents and compares the performance two multi-path topologies. Finally, section V presents our conclusion.

II. Related work

This section presents related work in three key areas for our solution: *Molecular Communication*, *Nucleic Acid Nanotechnology* and *Synthetic Biology*.

A. Molecular Communication using DNA

Molecular Communication is an emerging paradigm that seeks to use biological molecules and phenomena to encode and transport information. Research in this area tends to

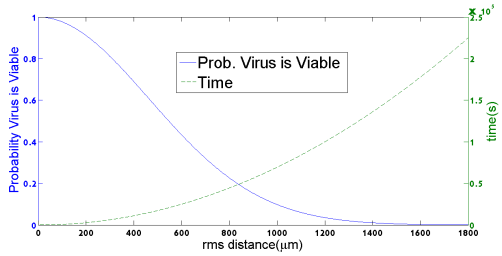

Fig. 1. Mean square displacement vs. survival probability(left axis) and average time(right axis) for a virus particle. Approximately 50% of virus particles decay before travelling 600μm from sender. 95% decay before reaching 1200μm. The displacement,d, is calculated in 2 dimensions using the equation $d^2 = 4Dt$ where d is mean square displacement, t is time and D is the diffusion coefficient($3.6\mu m^2/s$) and virus particle decay rate of 25/day(lentivirus)[14].

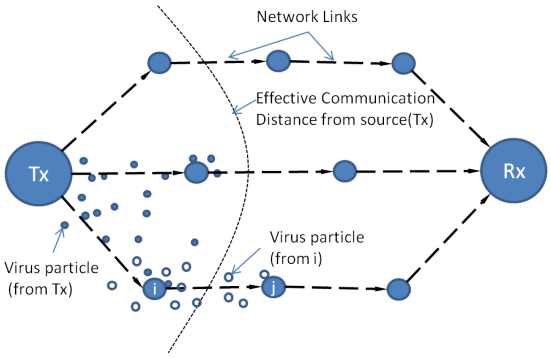

Fig. 2. Illustration of multi-path paths. The paths through the network are illustrated by dotted link lines.

exploit naturally occurring biological signalling mechanisms to design biological based communication solutions. For example, in [11] Gregori and Akyildiz explore the use bacteria to carry DNA plasmid messages. The authors use DNA as message molecules by encoding routing and data information sections in DNA strands. This is analogous to Protocol Data Units(PDUs) used in conventional data networks. The authors work includes analytical results for end-to-end communication, where bacteria are attracted and attach to a source nanomachine, loaded with DNA message molecule, and then propagate to and unloads the DNA message plasmid at the receiving nanomachine. In [11], Lio' and Balasubramaniam proposed a multi-hop routing mechanism that also utilises bacteria as a DNA message carrier for a network of nanodevices. Their proposed solution emulates *Delay Tolerant Networks (DTN)* found in mobile networks, where devices opportunistically encounter each other to pass DNA encoded messages.

While bacteria provide the ability to carry DNA based messages, the use of viruses has one significant benefit: Viruses can latch naturally onto cells to unload their payload molecules, which is beneficial for cell-based nanomachines (e.g. cells that are engineered to act as nanomachines).

B. Nucleic Acid Nanotechnology and Synthetic Biology

Nucleic acids are increasingly being used in nanotechnology. For example, artificially engineered nucleic molecules are used create and self assemble complex nanoscale structures[5] and machines[6]. Furthermore, simple algorithms such as an adder and subtractor[7] have been *programmed* using nucleic acids, utilising primarily the predictability and affinity of how nucleic acids bind to each other. For the most part, these applications operate *in vitro* and separate from the cell based genetic mechanisms. However, the creation of synthetic biology systems *in vivo* using existing biological mechanisms and genetic pathways provides the advantage of interfacing directly with other biological systems. For example, in [8], Xie et al show how in vivo "molecular computers" could re-use biological processes and create programmable units which implement biological computation. Similarly, in [9], Rinaudo et al use the *RNA interference (RNAi)* pathway and *short interfering RNAs(siRNAs)* to block/allow specific gene expression based on a set of molecular inputs, and in doing so implementing synthetic circuits in the cell. This provides a generalised technique of creating logic circuits that can be integrated to specific gene expression. In relation to information encoding in nucleic acids, in [4] the authors demonstrate rewritable nucleic acid based digital data storage in biological cells. By developing a rewritable addressable data module that switches between states in response to distinct inputs, the authors demonstrate data storage in DNA. In our work we intend to map networking properties using existing nucleic acid nanotechnology and synthetic biology methods to realise the functions necessary to create a multi-path virus based nanonetwork. The next section describes our proposed solution inspired by some of the works referenced in this section.

III. Proposed Solution

Our solution utilises virus based molecular communication between a transmitter and receiver device through an environment containing intermediary nodes which can replicate messages. As illustrated in fig. 1, virus propagation relies on suitable hosts within their effective communication range. The host cell must also expose complementary receptors on its surface for a virus to successfully attach. We focus on a simple multi-path design, *defined multi-path* ,whereby explicit paths are defined through the topology by using specific receptor and addressing pairs as illustrated in fig. 2. We now explain the use of RNAi pathway for virus control and develop a diffusion based model to analyse to performance of multi-path topology.

A. Virus and RNA Interference(RNAi)

Our research uses viruses to transport and transduce genetic information between biological cells. In [10] we presented a communication platform for biological nanodevices using artificially engineered cells, offloading the network layer and the physical communication layer to the cell. In this paper we re-use this approach and propose an application interface in the cell using a *RNAi* mediated genetic circuit. This allows

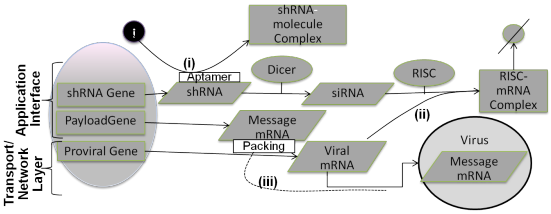

Fig. 3. Application interface using shRNA circuit taken from [10]. (i) The interfacing device interacts with the shRNA pathway via the aptamer portion of the shRNA. This leads to a disruption of the siRNA pathway, which prevents the formation of RISC/viral mRNA complex (ii). This will then lead to virus particle production (iii).

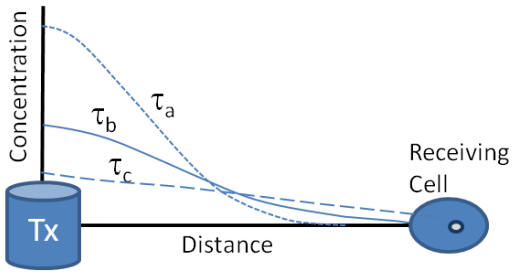

Fig. 4. Illustration of single network link. The transmitter releases virus quantity at τ_0, and the graph illustrates the resulting concentration gradient at times τ_a, τ_b and τ_c, ($\tau_a < \tau_b < \tau_c$). The virus particles begin to reach the receiving cell at $\tau = \tau_b$

the conceptual design of a biological communication circuit in the source cell that can control virus particles at the physical layer, and this is illustrated in Fig. 3. Based on work in [10] and [11], our solution uses aptamer fused *short hairpin RNAs(shRNA)* that have high affinity to chemical signals from interfacing nanodevices to control expression of proviral packing and addressing genes in the cell. The payload gene transcribes messenger RNA (mRNA) containing a packing nucleotide sequence which marks it for packing into a virus for transmission. The packed mRNA can have an address region and and message region in its nucleotide sequence. The address region of the mRNA molecule is analogous to the destination address field of an IP packet indicating the logical address of destination. This can be reverse translated in the receiving cell into a physical layer address which produces a virus that packs the replicated mRNA message molecule. The virus surface, in turn, is encoded with ligands/proteins that bind with receptors on the next hop node in the network.

B. Analytical Model

In this section we develop a deterministic analytical model for virus particle transmission between nodes. We consider the movement of virus particles a pure diffusion process [12] from the sender to the receiver. We model transmission in a two dimensional environment. The sender encodes messages in the form of mRNA that are encapsulated into virus particles. For simplicity, this is modeled as an impulse point source of Q particles. During an emission event, all virus particles encapsulate the same encoded RNA message. The concentration of particles at some distance r_c at time t from the sender is given by the solution to the diffusion equation in two dimensions for an instantaneous point source as follows [13]:

$$c(r_c, t) = \frac{Q}{4\pi Dt} exp(\frac{-r_c^2}{4Dt} - k_d t) \quad (1)$$

where D is the environment diffusion coefficient, c is the virus concentration, k_d is the virus particle decay rate, and t is time. We approximate the transmission channel as a set of discrete square locations, and assume all events occur at some discrete time $\tau_\eta = \eta\tau_{diff}$, where $\eta \in Z$, $0 \leq \eta \leq T$, and $\tau_{diff} = d_c^2/4D$, which is the average time it takes for a virus to diffuse a distance d_c in two dimensions. We can now express (1) as:

$$c_{ij}(\tau_\eta) = \frac{Q}{4\pi D\tau} exp(\frac{-r_{ij}^2}{4D\tau} - k_d\tau) \quad (2)$$

where $c_{ij}(\tau_\eta)$ is the concentration at the location containing node *j* at event time τ_η due to a release event from node *i* at time τ_0, and r_{ij} is the distance between node *i* and receiver location containing node *j*. Thus virus particle concentration for each location can be calculated as a function of spatial location and discrete time events. Solving (2) for several event times is shown in Fig. 4. Assuming virus particle concentration is spatially homogeneous in each location, then at some event time τ the expected number of absorbed virus particles for a transmission between nodes *i* and *j* can be approximated by:

$$V_{ij}(\tau) \approx k_{ab} Y_j c_{ij}(\tau) \tau_{diff} \quad (3)$$

Where Y_j is the receiver concentration in the location containing node *j* and k_{ab} is the rate of virus absorption. If we model the absorption of virus particles as a Poisson counting process [12], it follows that the probability of no absorption events occurring at τ is therefore:

$$p_{ij}(\tau) = exp(-V_{ij}(\tau)) \quad (4)$$

We assume that the first virus absorbed is successfully processed in the receiving node and that this will initiate forwarding of the virus particles in the intermediary nodes. Thus the probability of the first virus reception occurring at some event time τ_η is given by:

$$P_{ij}(\tau_\eta) = (1 - p_{ij}(\tau_\eta)) \prod_{\tau=\tau_0}^{\tau_{\eta-1}} p_{ij}(\tau_\eta) \quad (5)$$

where $p_{ij}(\tau_\eta)$ is the transmission failure probability during event time τ_η given by (4). We consider an explicitly defined multi-path routing topology consisting of independent paths such that, for each hop, a released virus can only attach to the next hop node for forward transmission . This can be achieved by addressing transmitted virus through the envelope gene using RNAi described in section III-A and is illustrated by the path lines in Fig. 2.

We define S as the set of all paths in the network from source to destination and each path s_i in S has M_{s_i} hops. We

define a set of corresponding link reliabilities for each path, $B_{s_i} = [\beta_{ij}, \beta_{jk}...\beta_{lm}]$ where β_{ij} is the reliability between nodes i and j and is calculated as follows:

$$\beta_{ij} = 1 - \prod_{\tau=\tau_0}^{\tau_T} p_{ij}(\tau) = 1 - exp(-\tau_{diff} k_{ab} Y_j \sum_{\tau=\tau_0}^{\tau_T} c_{ij}(\tau)) \tag{6}$$

In the case where all paths are disjoint then the overall network reliability (i.e. successful transmission over at least one path) is:

$$\beta_{MP} = 1 - \prod_{s_i \in S} (1 - \beta_{s_i}) \tag{7}$$

where β_{s_i} is the reliability of the path s_i in S and is calulcated as:

$$\beta_{s_i} = \prod_{\beta_{ij} \in B_{s_i}} \beta_{ij} \tag{8}$$

Assuming transmission across a link $i \rightarrow j$ is successful then the average transmission delay is the event time , τ_{ij}, such the cumulative probability of the first virus reception reaches half the overall link reliability:

$$\tau_{ij} = \{\tau_n : \sum_{\tau=\tau_0}^{\tau_n} P_{ij}(\tau) \approx \frac{\beta_{ij}}{2}\} \tag{9}$$

For each path, the average delay is:

$$\tau_{s_i} = (M_{s_i} - 1)\tau_l + \sum_{\tau \in T_{s_i}} \tau \tag{10}$$

where τ_l is the transmission latency at each node and $T_{s_i} = [\tau_{ij}, \tau_{jk}...\tau_{lm}]$ is the set of all transmission delays for each link in the path s_i. Thus the average transmission delay over all paths in the network is the sum of the individual average path delays multiplied by the respective path reliability from (8) and divided by the sum of all path reliabilities.

$$\tau_{mp} = \frac{1}{\sum_{s_i \in S} \beta_{s_i}} \sum_{s_i \in S} \beta_{s_i} \tau_{s_i} \tag{11}$$

The summation component in the left hand side of (6) indicates that maintaining a high virus concentration at the receiver location over time increases the reliability of a single link. Our expression for path reliability is also dependant on distance and time. It is clear that the reliability will decrease as the link distance increased. Furthermore, it will take a longer time to achieve maximum reliability for distant nodes and is illustrated in Fig. 4. If it is the case that a critical reliability, β_c, must be maintained for a particular link then the next hop must be within an effective radius r_e of the sender such that $\beta_{ij} >= \beta_c$ for all $r_{ij} <= r_e$. For a multi-path network, the effective radius could be used as a weight in routing algorithms whereby a nanodevice calculates the distance to nodes and only considers transmission to those within the effective communication distance. In [15], Moore et al measure distance between communicating nanodevices using Molecular Communication. However, calculating distance would require an additional computational capability in the respective nanodevices.

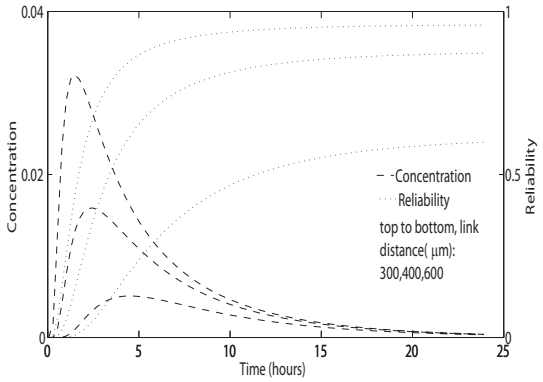

Fig. 5. Link reliability and virus particle concentration as a function of time for a 300, 400 and 600μm link and transmission of 3×10^4 virus particles. Note that this is the cumulative reliability calculated using (6). The reliability continues to increase over time when a significant virus concentration exists at the receiver.

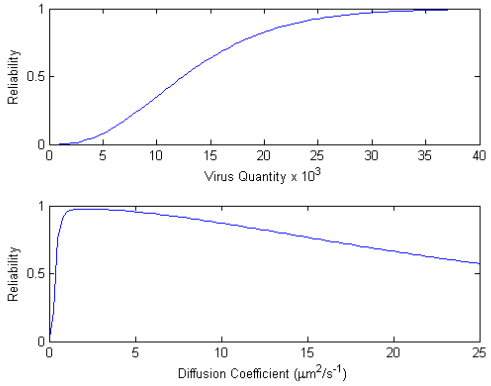

Fig. 6. Reliability vs. Released Virus Quantity (top) and Reliability vs. Diffusion Coefficient (bottom) for Topology 1 illustrated in fig.7 and the release of 3×10^4 virus particles. Diffusion coefficient of $3.6\mu m/s^{-1}$ used in top figure.

IV. Numerical Results and Discussion

Our numerical study focuses on multi-hop transmission between a source and destination device $1200\mu m$ apart in a channel with diffusion coefficient of $3.6\mu m^2 s^{-1}$(the diffusion coefficient for lentivirus in an aqueous environment[16]) unless otherwise stated. We assume the virus absorption rate by a host cell is $3.125\mu m^2 s^{-1}$[14] and a virus decay rate of 25/day[14]. The replication time for infected biological cells to emit progeny virus is in the order of days[17], so we assume intermediary nodes are engineered to release a burst of particles 24 hours after a reception event($\tau_l = 24h$). We also impose a constraint that all paths in the network are independent. Fig. 6 shows the effect of virus quantity and diffusion coefficient on reliability. Obviously end-to-end reliability of the multi-path topology is improved by increasing the virus quantity released, assuming other physical factors are fixed. In this case, reliability of greater than 0.95 can be achieved for released virus quantities in excess of 25×10^3

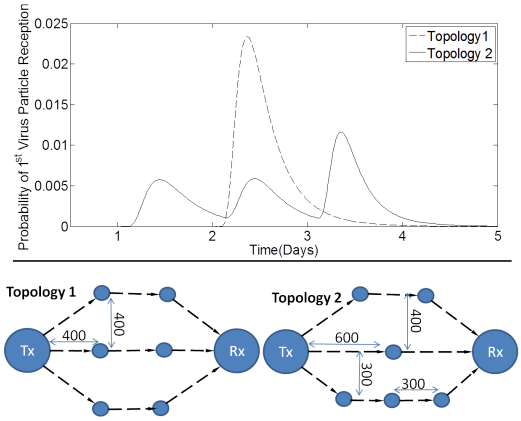

Fig. 7. Probability mass function of first successful particle transmission time over 1200μm (top) for two multipath topologies (bottom). All nodes only communicate with the nearest neighbour and all distance units are μm.

particles. Reliability could also be improved by using an acknowledgement protocol and this will be investigated in future work. As expected, reliability increases initially with the diffusion coefficient because a smaller proportion virus particles are decaying before they reach a viable host(fig. 6, bottom). The reliability reaches a maximum at D=2.5$\mu m^2 s^{-1}$, beyond which reliability begins to decrease with increasing diffusion coefficient. Larger diffusion coefficients do result in faster and further virus particle propagation but also disperse into the aqueous environment more rapidly. It is this dilution effect that begins to dominate for $D > 2.5\mu m^2 s^{-1}$. This results in a smaller concentration of virus particles being maintained over time at the receiver and consequently a lower probability of absorption. Thus reliability performance of multi-path network topologies is affected significantly by the physiochemical diffusion properties of the deployed environment.

The replication latency in each intermediary node significantly increases the end-to-end transmission time and end-to-end time variance. Fig. 7 illustrated the probability mass function(PMF) for first virus transmission time for two multipath topologies. The PMF for topology 1 exhibits a much tighter spectrum that that for topology 2. This is because virus replication latency is substantially larger than corresponding average link transmission delay calculated using (9) and topology 2 contains paths with various node counts. Therefore, increasing the number of nodes on a path will increase overall reliability but this is at the expense of increased end-to-end transmission time and increased end-to-end time variance. One approach to reduce end-to-end transmission time variance is for all redundant paths to have an identical hop count as in topology 1. This would facilitate better scheduling of messages in the network. On the other hand, if message scheduling and latency are not important, several redundant paths with various node counts could provide acceptable reliability, similar to Delay Tolerant Networks referred to in an earlier section, and may require the deployment of less nodes.

V. Conclusion

We have proposed an analytical model that can be used to calculate the reliability of multi-path virus nanonetworks. The numerical results indicates that particle dilution and diffusion speed of virus particles can have a significant and in some cases counter-intuitive effect on transmission delay and reliability. Furthermore, the replication latency in intermediary nodes dominates the overall end-to-end transmission delay. As a result, large transmission delay times will occur on paths containing many intermediary nodes. Our future work will examine how a simple implicit acknowledgement protocol, the number of paths, and channel encoding techniques can be used to improve the efficiency and reliability of virus based nanonetworks.

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A.8 Reliability and Delay Analysis of Multi-hop Virus-based Nanonetworks

In analysing the results from the paper in appendix A.7, we considered what protocols could be employed to improve reliability in virus-based nanonetworks. In this paper we proposed a single-path, multi-hop topology with implicit acknowledgement. We extended the analytical model in [65] to compare single-path and multi-path approaches. Furthermore, we compare the performance of artificial *defined-path* topologies to the naturally occurring random multi-path topologies of virus propagation.

Reliability and Delay Analysis of Multi-hop Virus-based Nanonetworks

Frank Walsh, Sasitharan Balasubramaniam, *Member, IEEE*

***Abstract*—Molecular communication is a new communication paradigm that allows nanomachines to communicate using biological mechanisms and/or components to transfer information (e.g. molecular diffusion, molecular motors). One possible approach for molecular communication is through the use of virus particles that act as carriers for nucleic acid-based information. This paper analyses multi-hop molecular nanonetworks that utilize virus particles as information carrier. The analysis examines the physiochemical and biological characteristics of virus particles such as diffusion, absorption, and decay and how they affect reliability of multi-hop transmission. The paper also analyzes the use of a simple implicit acknowledgement protocol for a single-path topology, and compare its performance to defined and random multi-path topologies that do not use acknowledgments. Numerical analysis shows that commensurate reliability is achievable for single-path with implicit acknowledgement and multi-path topologies. However, the single-path topology exhibits increased communication delay and more uncertain end-to-end communication time.**

***Index Terms*—Nano and molecular communication, nanonetworks, virus-based nanonetworks**

I. Introduction

The advancement of nanotechnology has led to development of miniature devices, which has brought new opportunities for various applications. One particular application domain that has benefited immensely from nanotechnology is in the health care domain. Example contributions of nanotechnology in health care include accurate analysis and detection of harmful diseases and drug transportation (e.g. through nano particles) to accurate locations within the tissues to terminate harmful diseases. These applications have been enabled by the miniaturized devices which can be placed in hard to access areas. The term *nanodevice* or *nanomachine* refers to a device composed of nanometer scale components ($10^{-9}m$) that possesses the ability to perform simple tasks [5]. While recent advancements have resulted in sophisticated devices, nanodevices have numerous limitations. For example, the limited processing capabilities of these devices lead to limited functionalities. However, these functionalities can be expanded when communication capabilities are integrated into nanodevices. In particular, communication between the devices can further extend the application base of nanotechnology. The field of *molecular communication* [9] aims to enable communication between nanodevices in a biological environment. In this new paradigm, information is transformed into molecules that utilize biological environments to transport information. Examples of molecular communication solutions that have been investigated includes calcium signaling [10], molecular diffusion [1][3], and bacteria communication [4][11]. These solutions are now being used to investigate molecular communication nanonetworks using molecular arrays [2] and body area nanonetworks [6].

However, developing networking capabilities between nanodevices poses a number of challenges [7][8]. First and foremost, communication between the devices is highly unreliable and suffers from long delay. This requires that communication protocols developed for nanodevices must consider these properties, and at the same time, the design of such protocols must fit and suit the biological process. Molecular communication network topology has a significant effect on several important factors such as reliability and efficiency. An acceptable level of robustness and redundancy must be present in the network to ensure that it can support critical services and continue to perform when parts of the network are temporarily down. These issues are not dissimilar to those faced in conventional sensor networks. However, nanonetworks must also take into account domain specific factors such as increased delay, particle decay, and the computational constraints of nanodevices.

In this paper, we propose the use of virus particles as information carrier for molecular communication. We consider virus particles as organic nano particles consisting of the following parts: a nucleic acid-based payload of either *DNA* or *RNA* (*Deoxyribonucleic Acid* and *Ribonucleic Acid*, respectively) information molecules, a protein coat that protects the information molecules, and a lipid envelope that encapsulates the protein coat. In particular our focus is on the capability of using virus particles to transport nucleic acid-based messages through a network topology. Viruses have a number of appealing properties that make them suitable for use in nanonetworks. These properties include the ability to encapsulate nucleic acid molecules and carry them to other distant cells. By latching onto cells, the virus can release the encapsulated message. This mechanism has made viruses useful for gene therapy applications and based on these characteristics we apply them as carriers for molecular communication. A particular functionality that we intend to investigate is multi-hop networking that could be achieved through virus-based nanonetworks. Since virus particles can only propagate to a certain distance, a relaying mechanism will be required for a network of nanomachines. We investigate multi-hop routing and how this is impacted for different topology shapes. In this paper we design and compare two approaches to topology design for virus-based nanonetworks. The first is a single-

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path topology with implicit acknowledgement. The second is redundant multi-path topology inspired by natural virus propagation mechanisms. We evaluate both topology design approaches in the context of transmission delay and reliability.

This paper is organized as follows: Section II presents the related work in molecular communication, in particular focusing on nanonetworks. Section III describes the key properties of viruses that are relevant to our proposed networking mechanisms between nanomachines. Section IV presents an overview of virus-based nanonetworks. Section V describes the analytical model for network links, single-path and multi-path topology in virus-based nanonetworks. Section VI presents and compares the performance for single-path and multi-path topologies. Finally, Section VII presents our conclusion.

II. Related Work

Since the introduction of molecular communication, a number of different solutions have been explored and developed. In this section, we will present some works that have investigated molecular communication from both a wireless and wired perspective.

A. Wireless Paradigm of Molecular Communication

Wireless devices communicate typically using radio waves that propagate through space. From a molecular communication perspective, a number of approaches have been investigated that mimic the propagation of radio waves using molecules. A good example is the diffusion process, which occurs in biological systems, where a node emits molecules that diffuse through the medium and slowly approach the receiver. In [10] Nakano and Liu investigated calcium signaling as a mode for molecular communication from an information theory perspective. This included the information transfer rate and its dependence on the concentration as well as distance of propagation. In [18] and [32], Pierobon and Akyildiz investigated the achievable end-to-end capacity using molecular diffusion. This included investigating the impact of noise and how this could affect the channel capacity. In [17], Gregori and Akyildiz proposed the use of bacteria as a carrier for molecular communication. Similarly to viruses, bacteria are able to carry genetic contents in the form of DNA, and are able to mobilize themselves towards a destination point. In [11], Balasubramaniam and Lio' proposed a multi-hop routing mechanism that utilizes bacteria as carrier for a network of nanodevices. The process mimics the mechanism of *Delay Tolerant Networks (DTN)* found in mobile networks, where devices opportunistically encounter each other to pass messages. While bacteria provide the ability to carry DNA-based messages, the use of viruses has one significant benefit: viruses can latch naturally onto cells to unload their nucleic acid payload, which is beneficial for cell-based nanomachines (e.g. cells that are programmed to act as nanomachines).

B. Wireline Paradigm of Molecular Communication

While wireless paradigms have gained the most popularity for molecular communication, there have also been approaches proposed for wireline paradigms. In [20] Enomoto et al. proposed creating artificial microtubules to connect between different nanomachines, which in turn mimics the wireline interconnection found in conventional communication networks. The transportation process is conducted through molecular motors that walk on the microtubules. In [22], Moritani et al. proposed vesicle-based nanotubes to interconnect devices. These nanotubes could in turn allow various types of molecules to glide between different nanomachines. The use of neurons has also been proposed for molecular communication. In [21], Balasubramaniam et al. designed an interface between nanodevice and neurons that could initiate signaling. The design also included a transmission scheduler between multiple devices connected to a neuronal network which ensured successful transmission with minimum interference.

Results contained in these related works often highlight the physiochemical and biological constraints of building molecular nanonetworks. For example, in [21] the refractory time for neurons places an upper limit on the signal throughput in a neuronal link. Similarly, Nakano and Liu [10] pointed out the effects of delay in replenishing intracellular calcium stores on the production of calcium signals. We now examine biological and physiochemical mechanisms of virus dynamics that both enable and constrain our design of virus-based nanonetworks.

III. Virus Characteristics

This section outlines key characteristics and properties of viruses used for multi-hop routing. As with all molecular communication networks, a protocol stack is required that provides the following layers: an application interface, network layer providing logical addressing, and a physical layer to provide a transmission mechanism for the biological channel. Many different virus types exist in biology, each adapted to a particular environment. However, all viruses share the following characteristics: a DNA or RNA core which contains the genetic information such as virus replication instructions for target cells and a protein coat (capsid) that encapsulates and protects the core. Furthermore, some virus types such as *lentivirus* have an outer lipid membrane or envelope that coats the capsid with ligands designed to attach the virus to target cell receptors. A simple illustration of a lentivirus lifecycle is shown in Fig. 1. We aim to use viruses as information carriers, reusing existing virus characteristics and mapping them to corresponding functions in a molecular communication protocol stack. Our proposed approach is a communication system that transports the information between different bionanomachines. The intermediary nodes are synthetic cells that have been engineered to forward messages to the next hop address in the similar way to intermediary nodes in wireless sensor networks. As we can see in Fig. 1, the destination does not continue to produce viral particles to take messages onto the next node, but instead goes through a reverse transcription to produce proteins that will be recognized by the attached bionanomachine or sink.

A. Viral-based Molecular Communication

Our research uses the ability of viruses to transport and transduce genetic information between biological cells. This

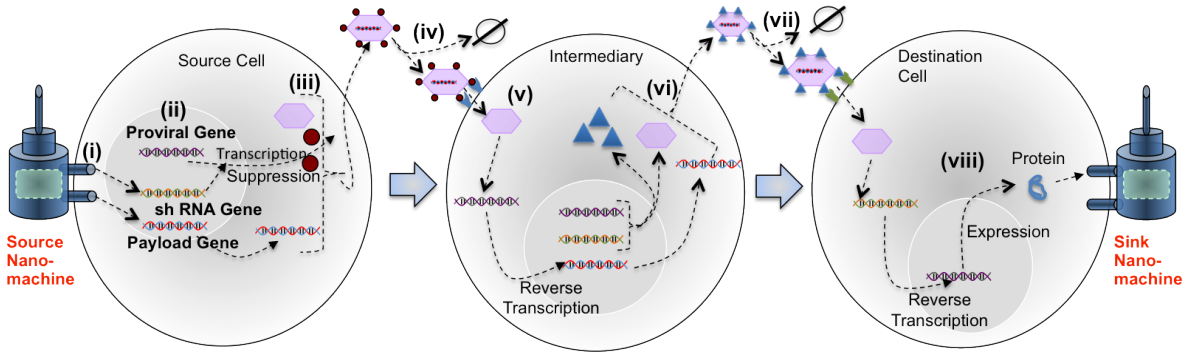

Fig. 1. Diagram showing stages of virus-based multi-hop nanonetwork. (i) The transduction of an extracellular event (e.g. from a nanodevice via application interface) inhibits the action of interfering RNA pathway and allows proviral gene expression. (ii) The Source cell is programmed to produce a genetic circuit. The cell transcribes proviral *messenger RNA(mRNA)* species. Simultaneously, the cell also transcribes shRNA that combines with DICER to silence proviral genes via siRNA pathway. (iii) The payload gene produces an encoded mRNA payloads which are packaged into the lentivirus and transmitted to extracellular space. (iv) The extracellular lentivirus envelope exposes ligands and either decays or bonds to the receptors on the target cell (i.e. next hop address). (v) Upon binding, the virus releases an encoded mRNA into the destination cell's cytoplasm, where the mRNA is processed by the cell. (vi) The translation of the mRNA in the intermediary node results in expression of new lentivirus for the next hop and also copies of the received mRNA payload molecule. (vii) The lentivirus is embedded with ligands for next hop destination and packs the duplicated mRNA payload. (viii) The lentivirus releases the mRNA payload for the destination cell, where the mRNA will be translated in the destination to actuate a response.

approach is similar to emerging therapies in the treatment of several human diseases where viral vectors are used to deliver an artificially engineered payload such as a DNA plasmid into specifically targeted biological cells [29]. While several types of viral vectors are currently used in biological research and medical science, this research focuses on *lentiviral vectors* due to their ability to target non-dividing cells and their current prevalence in biomedical research. Fig. 1 illustrates the routing of virus particles from source nanodevice to destination nanodevice via intermediary relay cells. We use the term *cell* and *node* interchangeably in the following sections since we are mapping the functions of a network node to biological cells. We focus on the network layer and physical layer aspects of these networks and do not consider DNA message encoding mechanisms. The reason for not considering the DNA message encoding mechanisms is because we assume that the nanomachine will have molecular computing capabilities, and can produce the DNA messages. Therefore, the output from the molecular computing process will be used by our proposed approach to transport to the receiving nano machine.

B. Virus Transmission

In our previous work [25] we proposed artificially engineered cells as communication platforms for nanodevices, allowing devices and sensors to interface to nanonetworks via biological cells, thus offloading the network and physical communication layers to the cell. We assume the application layer is contained in the nanodevice while an application interface with the cell is implemented using a *short interfering RNA (siRNA)* mediated genetic circuit. The use of siRNAs is a relatively new technique for gene expression control and can be used to silence specific aspects of viral gene expression and allow the conceptual design of a biological communication circuit in the source cell, and this is illustrated in Fig. 2. This approach is used to control proviral genes that are responsible for payload selection, packing and addressing of virus in the cell. A modular approach described in [25] and [26] uses aptamer fused *short hairpin RNAs (shRNA)* that have high affinity to chemical signals from interfacing nanodevices to control expression of proviral packing and addressing genes in the cell. The payload gene transcribes mRNA containing a packing nucleotide sequence which marks it for packing into a lentivirus for transmission. The packed mRNA can have an address region and a message region in its nucleotide sequence. The address region of the mRNA molecule is analogous to the destination address field of an IP packet indicating the logical address of the destination. This can be reverse translated into a physical layer address in the receiving cell which produces a virus that packs the replicated mRNA message molecule. The virus surface, in turn, is encoded with ligands/proteins that bind with receptors on the next hop cell in the network.

Our proposed virus-based nanonetwork is illustrated in Fig. 1, where we have an external nanomachine that interfaces to a cell and uses this as a transmitting platform. The nanonetwork also contains intermediate nanomachines that relay the viral particles from one nanomachine to the next. In this particular case, the intermediate nanomachines are synthetically engineered cells. Similar to the source nanomachine, the destination nanomachine is also interfaced to a cell, where the viral particles received by the cells will be passed onto the destination nanomachine. In Fig. 1, the source cell is engineered to contain the following proviral genes that translate to the biological components of a virus: a packing and envelope gene responsible for wrapping the DNA message in a protein coat that reflects the address, and a payload gene which produces genomic DNA/RNA payload (in this case the message molecule to be transmitted). As described above, the interfacing nanomachine controls the proviral genes in the source cell. Furthermore, as shown in [23] and [24], it is possible for the source cell to contain several envelope and payload genes which would give the ability to send multiple payloads to multiple destinations using a single source cell. In this paper we confine our study to one payload and envelope

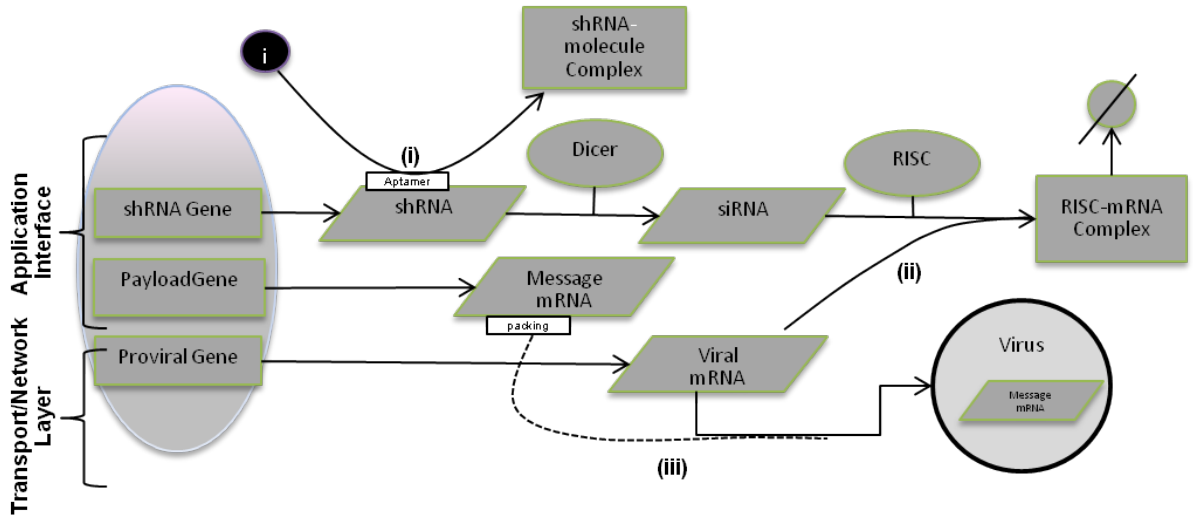

Fig. 2. Application interface using shRNA circuit taken from [25]. (i) The interfacing device interacts with the shRNA pathway via the aptamer portion of the shRNA. This leads to a disruption of the siRNA pathway, which prevents the formation of RISC/viral mRNA complex (ii). This will then lead to virus particle production (iii).

gene per cell. Once the required genes are unblocked, the virus particles are created and emitted into the extracellular space.

C. Virus Forwarding and Reception

Transmitted virus particles are received and processed by nodes with complimentary receptors with respect to their surface binding ligands. When a virus binds to a host it introduces an mRNA payload into the hosts cytoplasm where it is processed by the cell's internal machinery. As stated previously, the payload mRNA can have both addressing and data section which are processed separately: processing the address section results in the activation of gene that produces lentivirus for next hop transmission (i.e. encoded with surface ligands for next hop node). Processing the payload activates gene expression which is responsible for creating multiple copies of the mRNA message molecule complete with packing instructions for newly created lentivirus. Finally, virus particles are extruded out of the cell, essentially forwarding the mRNA payload to the next hop.

When the mRNA finally reaches the destination node, mRNA expression at the destination or sink results in activation of upper layer functions to process the payload section. This upper layer function could in turn be a molecular computing process that recognizes and interprets the address and the message from the payload. For experimental purposes, this could be activation of a gene that produces photo-luminous proteins which indicate successful transmission. As we can see in Fig. 1, the destination does not have the necessary proviral genes to forward viral particles but instead goes through a reverse transcription to produce proteins that will be recognized by the attached nanomachines.

D. Virus Cell Dynamics

Pearson et al. [30] proposed the following simple dynamic model for the absorption and release of virus particles from host cells:

TABLE I
VIRUS DIFFUSION PARAMETERS.

Symbol	Description	Value
D	Diffusion coefficient for virus in aqueous solution	$3.6\mu m^2 s^{-1}$ [14]
k_{ab}	Absorption rate of lentivirus by host cell	$3.125\mu m^2 s^{-1}$ [14]
k_d	Lentivirus decay rate	25/day [15]
d_c	Diameter of human T cell	10μm [16]

$$I \xrightarrow{Q} V + I \tag{1a}$$

$$V \xrightarrow{k_{ab}Y} I \tag{1b}$$

$$I \xrightarrow{\delta} \phi \tag{1c}$$

$$V \xrightarrow{k_d} \phi \tag{1d}$$

where V is the virus particle concentration, I is the infected cell concentration, Y is the target cell concentration, Q is virus extrusion rate from infected cells, k_{ab} is the absorption rate of virus particles by target cells, δ is the death rate of infected cells and k_d is the decay rate of virus particles. We use this model as a basis to design and simulate nodes in our multi-hop nanonetwork topologies, where viruses are used as information carriers. The above reactions can be used to model the message transmission (1a), message reception (1b), receiver loss (1c), and virus particle loss (1d). We assume that the virus infections do not cause the cell death described in 1c and that virus release is based on the continuous model as described in [30].

E. Virus Diffusion

Viruses are not living organism; they offer no form of self propulsion and require the internal genomic machinery of a

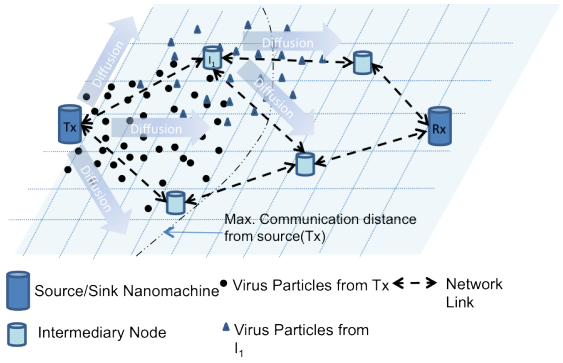

Fig. 3. Multi-path virus-based nanonetwork. Communication between the source and sink device (Tx and Rx, respectively) is routed via several intermediary nodes. Each network link involves: i) emission of virus particles, ii) transmission of virus particles across links via diffusion, and iii) adsorption at the receiver device.

host cell to replicate and propagate. We consider extracellular movement of virus particles over a single hop as a pure diffusion process [19] from the sender to the receiver. If virus particles are released in sufficient quantity, this process can be described using the standard diffusion equation:

$$\frac{\delta V}{\delta t} = D\frac{\delta^2 V}{\delta r^2} - k_d V \quad (2)$$

where D is the environment diffusion coefficient, V is the virus concentration at location r and k_d is the virus particle decay rate. For simplicity, we model transmission in a two dimensional environment as illustrated in Fig. 3, which approximates an infinite plane. The sender encodes messages in the form of mRNA that are encapsulated into virus particles whose emission is described by the function $Q(t)$ at the emitter. For simplicity, this is modeled as a point source. During an emission event, all virus particles encapsulate the same encoded mRNA message. The concentration of particles at location j at time t from a source at location i is given by integrating the solution to the diffusion equation for an instantaneous point source as follows [13]:

$$c(r_{ij},t) = \frac{1}{4\pi D}\int_0^t \frac{Q(t)}{t-t'}\exp\left(\frac{-r_{ij}^2}{4D(t-t')} - k_d(t-t')\right)dt' \quad (3)$$

where r_{ij} is the distance between location i and j, D is the diffusion coefficient for the virus particle in two dimensions, and t is time. This allows us to calculate the concentration of virus particles at a receiver location for a transmission described by the function $Q(t)$. Typical values for lentivirus parameters in 3 are shown in Table I.

IV. Virus Communication Nanonetworks

Since virus particles have limited spatial range, depending on the environment characteristics and virus concentration within the environment, intermediate nanomachines act as relay nodes that can receive certain virus particles and replicate them in much the same way as they do in nature. Our study focuses on reliability and delay in communication between

Fig. 4. Single-path (SP) topology. The message originates from Tx, and is passed along the relay nodes (A and B), before finally arriving at the destination node Rx.

distant nanomachines. In this section, we introduce two types of topologies that we have considered in our study: a single-path topology, and a redundant multi-path topology.

A. Single-path Topology

Single-path topology selects a set of intermediate nodes that have good link qualities and are used in sequence to forward particles to a destination nano machine. An example of a single-path is illustrated in Fig. 4. Transmission failure across each link could be handled using a simple implicit acknowledgement process as follows: consider transmission of one particle between nodes Tx and B via node A. During a transmission slot, Tx releases a quantity of virus particles, all containing the same message payload. When A successfully receives one particle from Tx, it proceeds to forward the particle to node B. During this period, Tx also listens for this forwarded particle from A and if it is not received within a time-out interval it is assumed that the transmission Tx to A has failed and Tx proceeds to retransmit the same quantity of virus particles. Each link failure will increase the overall communication delay by at least the time-out interval.

B. Multi-path Topology

Natural virus propagation through a medium is analogous to connectionless transmission in sensor networks. For example, virus emitting cells rely on the availability of suitable hosts within their communication range and the probability of successful virus propagation through a medium increases with the number of viable paths. We classify a viable path as having two hosts within the effective communication distance for each hop on that path. As seen in Fig. 1, in order for a virus to infect a target cell, the cell must expose complementary receptors on its surface. This gives two options for multi-path design: (i) *defined multi-path* whereby explicit paths are defined through the topology by using specific receptor and addressing pairs, or (ii) *random multi-path* whereby virus particles can be received and absorbed by all the nodes in the network.

V. Analytical Model

We now develop a deterministic model for reliability and transmission delay for virus-based nanonetworks based on the characteristics discussed in the previous sections. We develop initially a model for reliability and transmission delay over a single link between two nodes in a virus-based nanonetwork and then extend this analysis to develop models for single-path, defined multi-path and random multi-path topology.

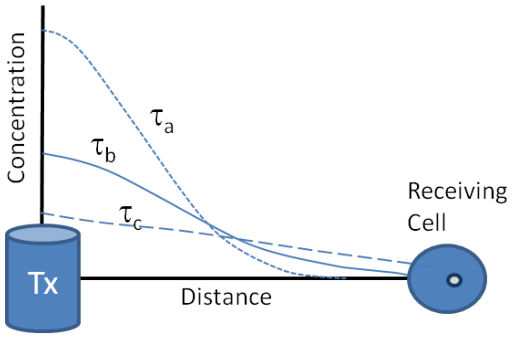

Fig. 5. Illustration of single network link. The transmitter releases virus quantity at τ_0, and graph illustrates the resulting concentration gradient at times τ_a, τ_b and τ_c, ($\tau_a < \tau_b < \tau_c$). The virus particles begin to reach the receiving cell at $\tau = \tau_b$

A. Network Link Analysis

We approximate the transmission channel as a set of discrete square locations, and assume all events occur at some discrete time $\tau_\eta = \eta\tau_{diff}$, where $\eta \in \mathbb{N}$, $0 \leq \eta \leq T$, τ_T is the maximum time an event can occur and $\tau_{diff} = d_c^2/4D$, which is the average time it takes for a virus to diffuse a distance d_c in two dimensions. We can now express (3) as:

$$c_{ij}(\eta) = \frac{1}{4\pi D}\sum_{x=0}^{\eta}\frac{Q(\tau_x)}{\tau_\eta - \tau_x}\exp\left(\frac{-r_{ij}^2}{4D(\tau_\eta - \tau_x)} - k_d(\tau_\eta - \tau_x)\right) \quad (4)$$

where $c_{ij}(\eta)$ is the concentration at the location containing node j at event time τ_η due to a release event from node i at time τ_0, and r_{ij} is the distance between node i and receiver location containing node j. Thus virus particle concentration for each location can be calculated as a function of spatial location and discrete time events. Solving (4) for several event times is shown in Fig. 5.

We model our receiver on (1b) and envision the receiver nanodevice as an artificially engineered cell, for example a modified human T cell of diameter 10μm [16]. If we examine the location containing node j and assume virus particle concentration is homogeneous in this location, then at some event time τ_η the expected number of absorbed virus particles for a transmission between nodes i and j can be approximated by:

$$I_{ij}(\eta) \approx k_{ab}Y_j c_{ij}(\eta)\tau_{diff} \quad (5)$$

Where Y_j is the quantity of nanomachines at location j (this is for both the relay and receiver nanomachine) and k_{ab} is the rate of virus absorption. Cell absorption of virus particles is modeled typically as a Poisson counting process [19] whereby the probability of α absorption events occurring at event time τ_η at location i is given by:

$$p_{ij}(\alpha,\eta) = \frac{I_{ij}^{\alpha}(\eta)\exp(-I_{ij}(\eta))}{\alpha!} \quad (6)$$

It follows that the probability of no absorption events ($\alpha = 0$) occurring at τ_η is therefore:

$$p_{ij}(0,\eta) = p_{ij}(\eta) = \exp(-I_{ij}(\eta)) \quad (7)$$

If virus emission begins at event time τ_0, then it follows that the reliability of a single link β_{ij} is:

$$\beta_{ij} = 1 - \prod_{\eta=0}^{T} p_{ij}(\eta) = 1 - \exp\left(-\tau_{diff}k_{ab}Y_j\sum_{\eta=0}^{T}c_{ij}(\eta)\right) \quad (8)$$

We are interested in the first reception event and assume that the first virus absorbed is successfully processed by the receiving node. We define a reception event as the first virus absorption at the receiver and this will initiate forwarding of the virus particles in the intermediary nodes. For a single hop, the probability of the first virus reception to occur at some event time τ_η is given by:

$$P_{ij}(\eta) = (1 - p_{ij}(\eta))\prod_{x=0}^{\eta-1}p_{ij}(x) \quad (9)$$

where $p_{ij}(\eta)$ is the transmission failure probability during event time τ_η given in (7). We can also calculate the average transmission time for a link ij as follows:

$$\tau_{AVE,ij} = \frac{1}{\beta_{ij}}\sum_{\eta=0}^{T}\eta P_{ij}(\eta) \quad (10)$$

It is apparent from the summation component of (8) that maintaining a high virus concentration at the receiver location over time increases the probability of a virus absorption event (i.e. link reliability). Our expression for link reliability is also a function of distance and time. It is also evident that reliability decreases with link distance and takes longer to achieve maximum reliability for distant nodes as illustrated in Fig. 5. In the event where a link must maintain a critical reliability, β_c, then the next hop must be within an effective radius r_e of the sender such that $\beta_{ij} >= \beta_c$ for all $r_{ij} <= r_e$. The effective radius could be used as a weight in routing algorithms whereby a nanodevice calculates the distance between its neighboring nodes and only considers transmission to those within the effective communication distance. This is used to calculate possible paths in random multi-path topologies in section V-C. Techniques to determine the distance between nodes in molecular communication based nanonetworks is explored in [12]. However, calculating distance would require an additional computational capability in each node.

B. Single-path Topology with Implicit Acknowledgment

We now expand the network link model to examine a simple single-path topology shown in Fig. 4. We define a path s connecting source and destination nodes as a set of M nodes starting with source node and ending with the receiver node. For example, $s = [1,3,4,6]$ represents a path from node 1 to node 6 via node 3 and node 4. We also define a set of corresponding link reliabilities, $B_s = [\beta_{1,3},\beta_{3,4},\beta_{4,6}]$ and link delays $T_s = [\tau_{AVE,1,3},\tau_{AVE,3,4},\tau_{AVE,4,6}]$. As in (4), we assume all events occur at some discrete time $\tau_\eta = \eta\tau_{diff}$. At each stage of particle transmission we assume the next hop and

previous hop are within the effective communication distance of each intermediary device. Each intermediary node replicates the original molecular communication after a cell propagation delay τ_l (i.e. releases the same number of particles at the same rate). Thus the path reliability (β_s) and the average transmission delay (τ_s) for a single-path from source to receiver with no link failures is:

$$\beta_s = \prod_{\beta \in B_s} \beta \tag{11}$$

$$\tau_s = (M-1)\tau_l + \sum_{\tau \in T_s} \tau \tag{12}$$

The single-path topology uses an implicit acknowledgement protocol as described in section IV-A. The average time for a successful implicit acknowledgement over a link ij is $(\tau_l + 2\tau_{AVE,ij})$ and the probability of receiving a successful acknowledgement is β_{ij}^2. We also wish to estimate a suitable time-out value for the acknowledgement protocol. The probability of the sender device receiving an acknowledgement at time τ_η over one hop with cell propagation delay τ_l is:

$$P_{ACK}(\eta) = \sum_{x=0}^{\eta-l} P_{ij}(x) P_{ij}(\eta - l - x) \tag{13}$$

where $\eta \geq l$. Using (13) we can also estimate a realistic retransmission time-out value $\tau_{ACK,ij}$ for a link such that the probability of acknowledgement failure for the link exceeds an acceptable threshold, $p_{ACK,c}$.

$$\tau_{ACK,ij} = min\left\{ \tau_x : 1 - \sum_{\eta=0}^{x} P_{ACK}(\eta) \geq p_{ACK,c} \ , \ x \leq T \right\} \tag{14}$$

Obviously single-path transmission delay increases with each link failure. For each link failure, the delay will increase at least by the corresponding link time-out value $\tau_{ACK,ij}$. An approach to implementing a time-out function could be based on the solutions proposed in [35] and [12], where an engineered chemical process in can act as a clock. The mechanism operates as follows: a source bionanomachine will release a quantity of virus particles and simultaneously start a chemical timer process which accumulates timer molecules. If no acknowledgement is detected and the source nanomachine detects a concentration of timer molecules greater than some predetermined threshold concentration, then the source bionanomachine will retransmit the message. The threshold concentration for the timer process would be calculated using the timeout value, $\tau_{ACK,ij}$. This implementation would also require a suitable biomolecular computing solution similar to those already referenced in [25] that would use the acknowledgement signal and the timer process concentration as inputs.

In a path s, we define f_{ij} as the number of retransmissions required to successfully transmit across link ij. The set of retransmissions for path s is $F_s = [f_{ij}, f_{jl} ....]$ and the total number of retransmissions is $f_s = \sum_{f \in F_s} f$. The probability of F_s retransmissions occurring during a transmission from source device to receiver is given by:

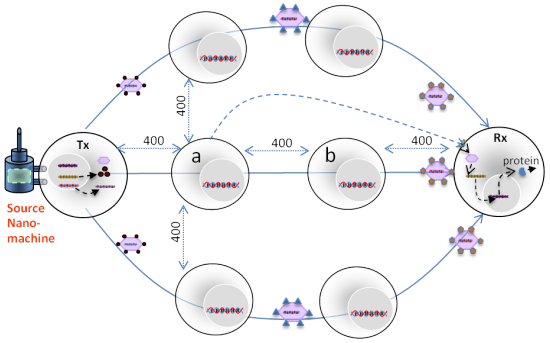

Fig. 6. Illustration of multi-path paths. The independent paths are illustrated by solid lines, while the dotted link indicates possible random paths. Distances are in μm and are used for numerical results in section VI

$$P_{s,F_s} = \prod_{\beta_{ij} \in B_s} (1-\beta_{ij})^{f_{ij}} \beta_{ij} \tag{15}$$

And the associated delay for path s and the link failure set F_s is:

$$\tau_{s,F_s} = \tau_s + \sum_{f_{ij} \in F_s} \tau_{ACK,ij} f_{ij} \tag{16}$$

We now can calculate analytically the reliability and delay for a single-path virus network with implicit acknowledgement.

C. Multi-path Topology

We initially consider an explicitly defined multi-path topology consisting of independent paths such that, for each hop, a released virus can only attach to the next hop node for forward transmission and the previous node for acknowledgement. This can be achieved by addressing transmitted virus through the envelope gene as described in section III-A and is illustrated by the solid path lines in Fig. 6.

1) Defined Multi-Path Topology with Independent Paths: We now define S as the set of all paths in the network and each path s_k in S has M_{s_k} hops. As in the single-path model, we define an set of corresponding link reliabilities for each path, $B_{s_k} = [\beta_{ij}, \beta_{jl}, \beta_{lm} ...]$ where β_{jl} is the reliability between nodes j and l.

In the case where all paths are disjoint then the overall network reliability (i.e. successful transmission over at least one path) is:

$$\beta_{MP} = 1 - \prod_{s_k \in S} (1 - \beta_{s_k}) \tag{17}$$

where β_{s_i} is calculated of each path in S using 11. Finally the average transmission delay is the sum of the individual path delays calculated using (12) multiplied by the respective path reliability from (11) and divided by the sum of all path reliabilities as follows:

$$\tau_{mp} = \frac{1}{\sum_{s_k \in S} \beta_{s_k}} \sum_{s_k \in S} \beta_{s_k} \tau_{s_k} \tag{18}$$

2) Random Multi-path topology with Dependent Paths: In the random multi-path topology, the virus particles operate as they would typically in nature. The characteristics of this approach have, as expected, a close resemblance to Gossip or Epidemic protocols [33] in conventional networks since both protocols are inspired by the characteristics of viral epidemics, albeit at an organism level (it is a case of coming full circle!). If we assume broadcast communication, where each virus-based message can be received by any node (i.e. all intermediary nodes have complementary receptors and can receive once they are within the effective communication distance of a transmitting node), then the virus can also have several dependent paths through the environment. For example Fig. 6 illustrates two dependent path $Tx \rightarrow a \rightarrow b \rightarrow Rx$ and $Tx \rightarrow a \rightarrow Rx$ whereby both have link $Tx \rightarrow a$ in common.

For comparison, we focus on a source and destination device in defined locations as in fig. 3. However, the intermediary nodes are distributed randomly in the 2D environment. The reliability and transmission delay is calculated as follows using Matlab [34]:

1 Calculate all possible node links, ij, using an effective communication radius described in section V-A and calculate the corresponding link reliability β_{ij} for each node pair using (8).
2 Create a set of all possible paths, S, from source node to end node using the path finding algorithm from [27] and sort in ascending order based on the hop count.
3 Calculate the conditional reliability of each path in S and then the end-to-end reliability using an implementation of the *SYREL* algorithm [28].
4 Create a subset of "effective paths" from *S* such that their combined reliability exceeds a predetermined reliability threshold. In this case we used .99 of the overall reliabilty calulated in step 3. This minimizes *pmf* calculations in the next step and removes long paths that make an insignificant contribution to overall reliability.
5 The *probability mass function (pmf)* for the first virus reception event is calculated for each path from step 4 as follows: (i) Using (9), the *pmf* for each path is calculated by successive convolutions of the *pmf* of each link ($P_{PMF,ij} = P_{ij}/\beta ij$) in the path. (ii) Via the principle of superposition, the path *pmf*s are combined to calculate the overall *pmf* for first virus reception event at the receiver.

This analysis can be used to calculate the *pmf* of first virus particle received at the receiver and is used in the next section (fig. 11) to illustrate the *predictability* of the first reception event.

VI. Numerical Results

We now analyze reliability and communication delay for a single link by varying link distance and diffusion coefficient. We then extend this to obtain the reliability and transmission delay for multi-hop routing between a source and destination device using single-path, defined multi-path, and random multi-path virus-based nanonetworks. For all multi-hop analyses, we examine communication between a source and destination device with fixed location $1200\mu m$ apart using each topology.

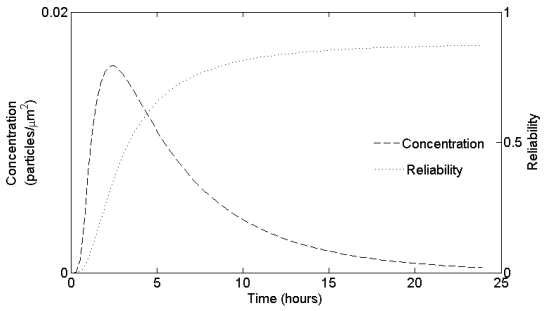

Fig. 7. Link reliability and virus particle concentration (virus particles/μm^2) as a function of time for a $400\mu m$ link and transmission of $3x10^4$ virus particles. Note that this is the cumulative reliability calculated using (8). The reliability continues to increase as long as a significant virus concentration exists at the receiver.

A. Single link

The calculation of virus particle concentration and associated link failure for a location set at 400μm from a sender is shown in Fig. 7. It is possible to visualize the virus particle propagation as a decaying wave front moving away from the transmission source resulting in the virus concentration profile illustrated in Fig. 7. Assuming the virus quantity released at the sender is fixed, then the virus dynamics as it moves away from a sender and encounters a receiver is constrained by the following physical and biological parameters: diffusion coefficient, distance from the sender, and virus decay. As already stated, the virus decay combined with the diffusion coefficient creates a theoretical maximum communication distance and can be calculated based on diffusion. From a communication network perspective, we are particularly interested in the reliability and transmission delay associated with a given link. Using (10) we calculate the effective link distance and the associated transmission delay for a link using a reliability threshold. The reliability threshold is the lowest average reliability permitted for a link. We illustrate this by plotting the average transmission delay and maximum link distance as a function of threshold reliability for a link. Analysis of Fig. 8 shows significant increase in transmission delay to achieve reliability greater than 0.7. Also, the gap in transmission delay between different link distances for the same threshold becomes wider as the reliability threshold goes towards 1. This is caused by the relatively longer time virus particles take to diffuse to outlying locations and also the dilution effect of the virus quantity as it spreads out from its source. For example, the difference in transmission delay to achieve reliability greater than 0.5 for a 100μm and 300μm link is 1.3 hours. The difference to achieve reliability greater than 0.9 for the same distances is 3.9 hours. This suggests that to create multi-hop networks with low delay and high reliability a redundant multi-path approach would increase the throughput time.

The effect of the diffusion coefficient on link distance is illustrated in Fig. 8(b). The behavior of single links in environments with a large diffusion coefficient shows interesting

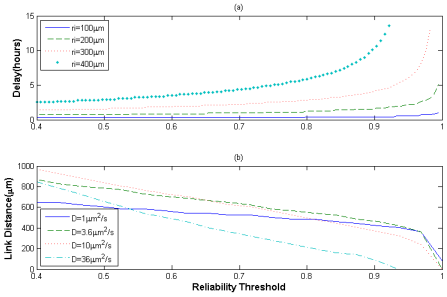

Fig. 8. Transmission delay (a) and Effective Link Distance (b) vs. Reliability Threshold for a single link. The quantity of virus Q(0) = 30000 and the link distance r_i = 400μm is used in (b). Other parameters are from Table I.

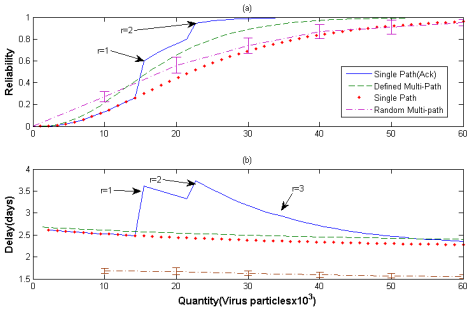

Fig. 9. Reliability (a) and Average Delay (b) vs. Virus Quantity released during transmission events. The plot is annotated with corresponding maximum number of retransmissions (r) used in single-path(Ack) calculations required to match the expected number of transmission in the defined multi-path.

results. Intuitively one would expect that faster diffusion would result in less transmission delay times and increased effective link distances for a given reliability threshold. However, calculating the effective communication distance for several diffusion coefficients suggests the opposite: that environments with higher diffusion coefficients have shorter effective communication distances. For example, effective link distance to achieve reliability greater than 0.9 is 500μm and 350μm for diffusion coefficients of $3.6\mu m^2 s^{-1}$ and $10\mu m^2 s^{-1}$, respectively. The larger diffusion coefficient does result in faster and further virus propagation, but with less concentrated molecules being maintained over time at the receiver and consequently lower probability of absorption. Thus reliability performance of multi-hop network topologies is affected significantly by the physiochemical diffusion properties of the deployed environment. We now analyze the design of single-path, defined multi-path and random multi-path topology models using the single link model as a basis.

B. Multi-Hop Topologies

We now compare the performance of the multi-path and single-path analytical models presented in Section IV. For single-path topology, we use the design shown in fig. 4 with each link distance set at 400μm. For the defined multi-path topology we use the paths and link distances shown by the solid lines in fig. 6.

Applications that will use this type of network will not have an indefinite time period to propagate molecules. Therefore, for a single-path with acknowledgement, we set a maximum number of single-path retransmissions. We also wish to compare accurately both single and multi-path approaches. Therefore, we set a maximum number of retransmissions, r, such that the number of transmission that can occur in the single-path topology is equal to the expected number of transmission in the defined multi-path scenario as follows:

$$r = 1 - M_s + \sum_{s_k \in S} \sum_{\beta_{ij} \in \beta_{s_k}} \beta_{ij}(h_{ij} - 1) \qquad (19)$$

Where β_{s_k} is the set of link reliabilities in path s_k, M_s is the number of hops in the single-path topology, and h_{ij} is the hop index for link $i-j$ in path s_k. For example, in path s_k=[1 2 3 4], $h_{s_k,12} = 1$ (first hop), $h_{s_k,23} = 2$ (second hop). Our numerical study focuses on multi-hop transmission between a source and destination device 1200μm apart in a channel with diffusion coefficient of $3.6\mu m^2 s^{-1}$. We also impose a constraint that communication is between neighbors and, initially, all paths in the network are independent. Successful particle delivery with respect to released virus quantity for single-path, multi-path and single-path with implicit acknowledgement and number of retries r calculated using (19) is shown in Fig. 9. The single-path without acknowledgement is also shown for comparison purposes. The Fig. 9 plot annotations indicate changes to single-path maximum retransmissions, r, required to match the number of transmissions that occur in the multi-path topology. It is apparent from Fig. 9 that the reliability of multi-path topology is improved by increasing the virus quantity, assuming other physical factors are fixed. Single-path reliability can also be improved by increasing the number of retransmissions allowed per message. However, as indicated in (13), the retransmissions significantly increase the average delay in transmission and this is illustrated in Fig. 9 (b) and fig. 10. This is due predominantly to the delay associated with each retransmission. Fig. 10 (a) shows superior average delay for a 2 hop single-path compared to 3 hop and 4 hop however, this is at the expense of inferior reliability. Reliability is improved by increasing the maximum number of retransmissions however this also increases the average delay time (fig. 10 (b)). We base our estimation of propagation delay, τ_l, on time it takes an infected cell to emit replicated virus. It is substantially larger than the corresponding hop transmission delay, $\tau_{x,ij}$ calculated in Section IV. The corresponding approach in multi-path topologies is to create more paths to the receiver. Doing so does not exhibit such dramatic differences in delay times and any delay time changes would be due to diffusion-based transmission delay already discussed in the single-path model.

We also include results for a random multi-path solution. In this case, source and destination nodes are in the same fixed locations, and we distribute the intermediary nodes randomly in a 1200μm x 1200μm area and do not impose any routing restrictions as described in section V-C1. The results indicate that this topology provides improved transmission delay compared to other topologies. This is due to the predominance of short two hop paths through the environment. However, reliability performance is less than the multi-path and single-

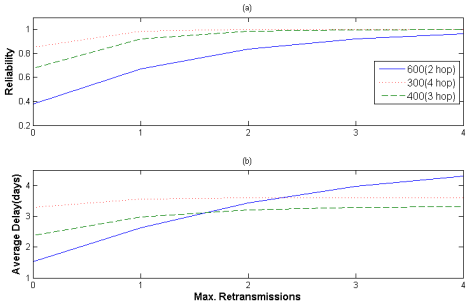

Fig. 10. Reliability (a) and Average delay (b) for successful transmission over single-path of 1200μm with 1, 2 and 3 intermediary nodes. All nodes are equidistant and only communicate with the nearest neighbors.

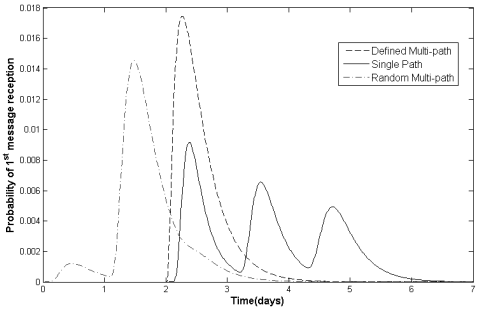

Fig. 11. Probability mass function for single-path, defined multi-path and random multi-path topologies. The random topology uses an identical number of intermediary nodes as defined multi-path. The random nodes are distributed in a simulation area of 1200μm by 1200μm. Each node releases $3x10^4$ particles per release event.

path with acknowledgement topologies, particularly for higher virus quantity emissions.

Finally, we examine the predictability of a successful message reception event at the destination device. Fig. 11 shows the *pmf* of time taken for the first message reception event at the destination. The *pmf* for the single-path approach with implicit acknowledgement is spread over a longer time compared to our multi-path approach. This is due to the high probability of retransmissions being required to attain the threshold reliability. The spikes in the plot for single-path correspond to message reception after zero, one and two retransmissions, respectively, as time increases. The defined multi-path topology, where each path has an identical number of hops as illustrated in fig. 6, exhibits a tighter *pmf* profile and, therefore, exhibits a more predictable performance for message delivery.

Clearly the defined multi-path solution appears to be more desirable in terms of minimizing delay and maximizing predictability. Also, it may be the case that multi-path solution might be a simpler solution to realize physically as it does not require the computational mechanisms to implement the implicit acknowledgement protocol. On the other hand, the single-path topology requires fewer relay nodes and the implicit acknowledgement solution does provide a *best of both worlds* in allowing reliability to be tailored by adjusting the maximum allowed number of retransmissions. For example, as discussed in section IV, changes in the diffusion coefficient can significantly affect reliability. Implicit acknowledgement allows the source to destination reliability to be re-programmed through the communication protocol by adjusting the maximum number of retransmissions. This would be of benefit, particularly, if the virus quantity released during a transmission event is a biological constant that cannot be modulated apart from on/off process described in Section III.

C. Comparison to other molecular communication solutions

The virus-based nanonetwork proposed in this paper is diffusion-based similar to those proposed in [1] and [3]. The difference is that we have selected one specific biological component for the diffusion (viruses) and these works do not cover the specific scenarios that we use in this paper (multi-path and single-path with acknowledgement). However, the bacteria model proposed in [4] and [11] can be compared with the virus model proposed in this paper. Based on the delay of the single link in fig. 8, for a distance of 200μm, the virus propagation takes approximately 5 hours. This is slightly faster than the bacteria communication model of [11] where a single link took approximately 4.9 hours for 180μm (however, in the case of bacteria communication only 30 bacteria were emitted, which is much lower than the quantity of virus emitted in this paper). Based on the delay and reliability pattern, we can observe that a similar delay pattern to multi-hop bacteria nanonetworks is observed [11]. The reason for this similarity is due to the infection process of virus on cells which increases the delay, and this is similar to the conjugation process in bacteria.

VII. Conclusion

The physiochemical and biological characteristics of virus-based nanonetworks combined with heterogeneous deployment environments pose challenges to the development of communication networks to transport data from the source to destination. Our analysis of a single link in virus-based nanonetworks shows that particle dilution and diffusion speed of virus particles can have a significant and in some cases counter-intuitive effect on transmission delay and reliability. Our numerical results show that equivalent reliability is possible in single-path with implicit acknowledgement and defined multi-path topologies for virus-based nanonetworks. However, the large replication delay relative to channel transmission delay means that single-path with implicit acknowledgement and random multi-path topologies introduce more uncertainty for source to destination communication time compared to defined multi-path topology. Furthermore, the analysis indicates that replication delay in intermediary nodes dominates overall transmission delay in multi-hop virus-based nanonetworks. However, this inherent delay can be offset by the ability to encode large amounts of information in DNA payload molecules [31]. Our future work will examine how different parameters such as the number of paths and channel encoding techniques can be used to improve the efficiency and reliability of virus-based nanonetworks.

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