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Molecular Communication for Nanomachines Using Intercellular Calcium Signaling

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***Abstract* — Molecular communication is engineered biological communication (e.g., cell-to-cell signaling) that allows nanomachines (e.g., engineered organisms, artificial devices) to communicate through chemical signals in an aqueous environment. This paper describes the design of a molecular communication system based on intercellular calcium signaling networks. This paper also describes possible functionalities (e.g., signal switching and aggregation) that may be achieved in such networks.**

***Index Terms* — molecular communication, nanomachine communication, synthetic biology**

I. Introduction

Communication plays a critical role in a broad range of nanoscale applications from nanoscale computing, sensing to nanomedicine. Communication provides a means by which nanomachines (e.g., engineered organisms or artificial devices) coordinately perform tasks that cannot be accomplished by a single nanomachine. For example, nanomachines that can function as basic logic gates [8] may perform distributed computing through communication; nano medical machines [7] with communication capabilities may perform coordinated monitoring of human health.

It is known that living cells in various tissues and organisms utilize numerous mechanisms to establish cell-cell communications. Existing cell-cell communication mechanisms may be applicable to designing controlled communication network systems between nanomachines [5]. In addition, current molecular engineering technologies may allow modification of biological components to architect components that are suitable for communication network systems between nanomachines and provides novel functionalities such as signal amplification and switching mechanisms.

This paper describes the type of the molecular communication systems in which nanomachines communicate with each other on a signaling network in an aqueous environment (Figure 1). Nanomachines are engineered organisms whose behavior is programmed to perform applications (e.g., coordinated sensing). The signaling network consists of networked nodes (i.e., components capable of transmitting or relaying a communication) providing a shared medium for nanomachines to communicate with each other. The potential models of a signaling network can be engineered from commonly used cell lines, such as gap junction transfected HeLa cells [6]. Some mutations will be introduced into the cells in order for nanomachines to achieve particular networking (e.g., broadcast, directed signaling).

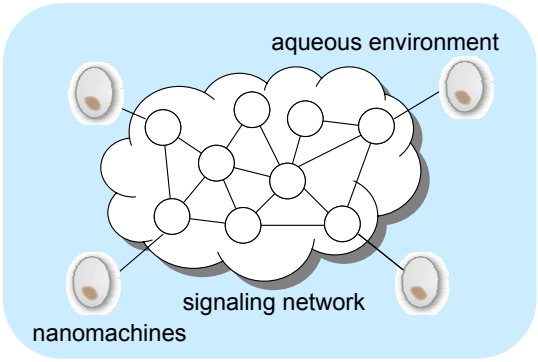

Figure 1: A molecular communication

To illustrate how communication is done in the system described in Figure 1, consider the following example. Sender nanomachines sense chemical substances in the environment and initiate a signaling by stimulating neighboring networked nodes (e.g., cells). The stimulated nodes then broadcast signals to their neighboring nodes. The receiver nanomachines receiving the node-mediated signals react to the incoming signals and initiate processes such as secreting chemicals, (e.g., cytokines), producing motion (e.g., through polymerization of actin filaments), and emitting light (e.g., through GFP expression).

In the following sections, we first describe calcium signaling, one type of cell-cell communication that is widely used by multi-cellular organisms. We then discuss possible design and implementation of a signaling

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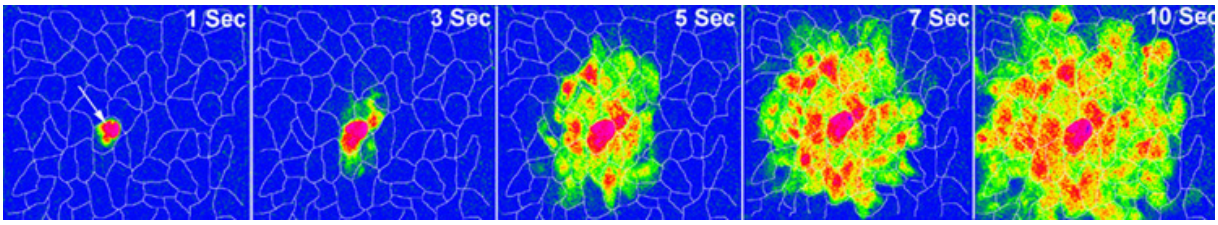

Figure 2: Calcium signaling among epithelial cells

network by exploiting the nature of cell-cell communication.

II. CALCIUM SIGNALING

a) Ca^{2+} - universal second messenger

In multi-cellular organisms, calcium ions (Ca^{2+}) commonly act as an intracellular (secondary messenger) to regulate various cellular activities (e.g., chemical secretion, contraction, proliferation, and death) [9]. For the generation of Ca^{2+} signals, cells depend on the large electrochemical gradient of Ca^{2+} across the plasma membrane (termed Ca^{2+} influx), and/or across the endoplasmic reticulum membrane. Information is precisely encoded on a frequency and amplitude of concentration changes [1] often referred to as Ca^{2+} spikes and oscillation. For example, differential gene activation occurs in response to the amplitude of a Ca^{2+} spike. Enzymatic activity of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) depends on the frequency of Ca^{2+} oscillation.

b) Ca^{2+} wave propagation

The increased cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) propagates cell to cell known as an intercellular Ca^{2+} wave. The intercellular Ca^{2+} waves have been studied in many cell types including non-excitable cells (e.g., epithelial cells as shown in Figure 2). For example, in resting states of glial cells, $[Ca^{2+}]_i$ ranges from 30 - 150 nM. Upon mechanically or chemically stimulated, $[Ca^{2+}]_i$ increases up to hundreds of nM or several μM within milliseconds [2]. This change propagates from cell to cell. The increased $[Ca^{2+}]_i$, after a refractory period, is restored to its resting state through the use of several Ca^{2+} pumps and cellular organelles (e.g., endoplasmic reticulum) that act as Ca^{2+} stores.

Ca^{2+} wave propagation can be mediated by two different pathways; internal pathway and/or external pathway [3]. The following briefly describes the two mechanisms that propagate Ca^{2+} waves between cells.

Intercellular Ca^{2+} wave propagation observed in some astrycotes is mediated by gap junctions (Figure 3) that

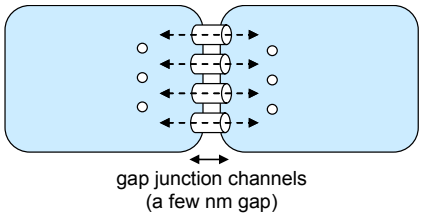

Figure 3: gap junctions

directly connect the interior of one cell and that of another. In this model, a stimulated cell produces a secondary messenger, inositol 1,4,5-triphosphate (IP_3), which leads to Ca^{2+} release from IP_3-sensitive Ca^{2+} stores such as endoplasmic reticulum (ER) of the stimulated cell. The produced IP3 diffuses through gap junctions to neighboring cells that triggers Ca^{2+} release from ER of the neighboring cells, resulting in increasing $[Ca^{2+}]_i$. The diffusion of IP3 continues, thus propagating Ca^{2+} waves over some number of cells.

Intercellular Ca^{2+} waves are also mediated by diffusion of signals through an external signaling pathway that involve extra-junctional connexons [3]. For instance, astrocytes and osteocytes cells secrete ATP into the extracellular environment. ATP then diffuses and binds to P_2 purinergic receptors on neighboring cells. This raises $[Ca^{2+}]_i$ of the cells and also activates ATP-releasing channels that release ATP into the extracellular environment, thereby propagating Ca^{2+} waves.

c) Gap junctions

A gap junction that plays an important role in possibly both types of Ca^{2+} wave propagation, consists of integral proteins called connexons, which is made of six four-pass transmembrane proteins, called connexins. There are over 20 connexins reported, and different combination creates a channel with different properties (e.g., different permeability). Gap junctions normally allow the passage of small molecules (<1000Da) (e.g., inorganic ions, IP3, cyclic AMP, cyclic GMP) between cells, and between a cell and the extracellular environment. Gap junctions adaptively vary the permeability (i.e., a probability that the channel is open) depending on various internal and external factors including cytosolic Ca^{2+} concentration,

transjunctional and transmembrane potential, connexin phosphorylation, electromagnetic fields, temperature and pH level in the environment.

III. NETWORK DESIGN

In the following, we discuss the design of molecular communication that uses Ca^{2+} signaling described in Section II-b. Figure 4 presents a schematic representation of molecular communication through gap junction mediated diffusion of intracellular molecules (e.g., IP_3). A sender nanomachine (marked S) interacts with a neighboring cell to initiate Ca^{2+} signaling (This process is called *encoding*.) The increased Ca^{2+} then propagates from cell to cell based on the mechanism explained in Section II-b. A receiver nanomachine (marked R) detects and reacts to the increased $[Ca^{2+}]_i$ of a neighboring cell. (This process is called *decoding*.)

In the following, we first describe encoding and decoding. We then describe possible functionalities such as signal aggregation and switching mechanisms that can be implemented on the signaling network.

a) Encoding: initiating signaling

Sender nanomachines initiate signaling by stimulating neighboring cells leading to the generation of propagating Ca^{2+} waves. The nature of generated Ca^{2+} waves may vary depending on types, duration and strength of the stimulation, and may affect whether Ca^{2+} waves reach neighboring cells of receiver nanomachines and whether the receiver nanomachines react to the waves or not.

In the case where sender nanomachines have direct access to the cytosol of neighboring cells (e.g., sender nanomachines and cells are forming gap junctions), senders may broadcast IP_3 to neighboring cells. For example, sender nanomachines with mechano-receptors, upon detecting the environmental changes, generate IP_3 and broadcast it to their neighboring cells. This triggers Ca^{2+} waves in the neighboring cells, which then propagate through a signaling network toward receivers.

In the case where sender nanomachines do not have access to the cytosol of cells, the senders may initiate signaling by releasing agonistic substances to the environment. The released agonists bind to membrane receptors of neighboring cells and activate G-protein receptors on the neighboring cells eventually leading to the generation of Ca^{2+} waves.

b) Decoding: reacting to signals

Receiver nanomachines detect and react to $[Ca^{2+}]_i$ of neighboring cells. Detection of or reaction to $[Ca^{2+}]_i$ of neighing cells may be performed directly or indirectly.

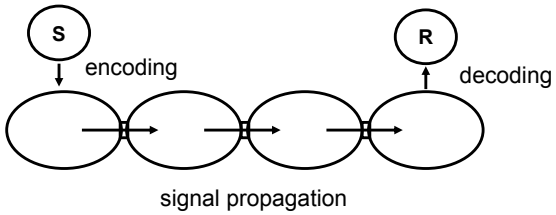

Figure 4: Molecular communication through gap junction mediated diffusion of intracellular molecules

In the case of direct detection, receiver nanomachines may establish gap junctions with the neighboring cells, allowing receiver nanomachines to directly react to $[Ca^{2+}]_i$ of neighboring cells. For example, receiver nanomachines may be engineered using Ca^{2+} sensitive molecules (e.g., calmodulin, troponin C, calpain, recoeverin) and produce a reaction according to influx of Ca^{2+} through gap junctions. Direct reaction to $[Ca^{2+}]_i$ may also be possible by receiver nanomachines with Ca^{2+} sensors that react to extrusion of Ca^{2+} from the neighboring cells if neighboring cells can release Ca^{2+} (e.g., through calcium pumps) into the environment when $[Ca^{2+}]_i$ is high.

In the case of indirect detection, receiver nanomachines may sense cellular activities (morphological changes, chemical secretion, light emission) that results from the $[Ca^{2+}]_i$ change of neighboring cells, and produce a reaction.

c) Signal propagation

Signal propagation can be broadcasted or directed depending on the type of signaling network formed. For instance, a signaling network can be a broadcast medium when a network consists of cells that express the same type of gap junctions with identical permeability. In this network, Ca^{2+} waves are indiscriminately broadcasted to neighboring cells as shown in Figure 2. Alternatively, signaling can be directed if a network is formed by cells that express gap junctions with different permeability. In this case, Ca^{2+} waves propagate selectively toward certain directions because signals diffuse through gap junctions that are more permeable to the signals.

Since gap junctions change the permeability according to various factors as briefly introduced in Section II-c, a signaling pathway (e.g., whether broadcasted or directed) can be dynamically controlled. Figure 5 illustrates a design of *cell switch* that allows controlled signaling by dynamically changing the permeability of gap junctions. In this figure, the cell switch that expresses three types of connexins, Cx-X, Cx-Y, Cx-Z, is connected with three cells, X, Y, Z that expresses Cx-X, Cx-Y, Cx-Z, respectively. For simplicity, it is assumed that a gap junction can be established only between the same types

of connexin. Shown in this figure is a case where an external signal is applied to the cell switch, which changes the permeability of a gap junction made of Cx-X, and thus signals propagating from cell Z diffuse only to cell Y. Such switching may be engineered using an external signal that activates a specific kinase (such as PKC, PKA) inside a cell switch, which can phosphorylate a specific type of connexin (i.e., Cx-X in this case) that leads to the decreased permeability of gap junctions consisting of the connexin.

Other possible functionalities that can be achieved on a signaling network include signal aggregation. Figure 6 illustrates how signal aggregation can occur on a network of cells. As explained in Section II-b, Ca^{2+} waves are often mediated by IP_3 that diffuses from cell to cell while degrading during diffusion. To enable signal aggregation, cells may be patterned in such a way that, when a single sender (either S1 or S2) stimulates a cell, the amount of IP_3 generated in the cell is not enough to reach a neighboring cell of receiver R; but when both of two senders S1 and S2 stimulate its neighboring cells, the combined amount of IP_3 is enough to reach a neighboring cell of receiver R and increase $[Ca^{2+}]_i$ of the cell.

IV. CURRENT STATUS

This paper presents our initial designs of a molecular communication system that exploits intercellular Ca^{2+} wave propagation. Our next step is to identify materials (e.g., cell types) to create the designed system and mechanisms. A signaling network using intercellular Ca^{2+} wave propagation may be engineered using cell cultures that form gap junctions, or connexin-transfected cells that are capable of cell-cell communication through gap junctions. A possible choice is transfected HeLa cells [6] as they are adherent cells that grow and form a network in a self-assembled manner.

We are planning to conduct both experiments and computer simulation in order to investigate the feasibility of the designed molecular communication system as well as to identify key communication characteristics of the designed systems.

ACKNOWLEDGEMENTS

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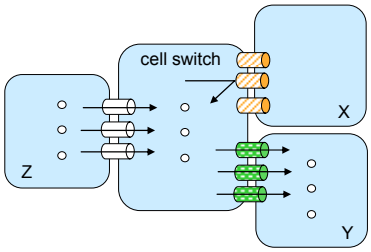

Figure 5: Signal switching

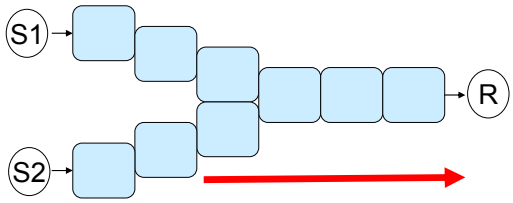

Figure 6: Signal aggregation

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