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# Model for biological communication in a nanofabricated cell-mimic driven by stochastic resonance

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ABSTRACT

Cells offer natural examples of highly efficient networks of nanomachines. Accordingly, both intracellular and intercellular communication mechanisms in nature are looked to as a source of inspiration and instruction for engineered nanocommunication. Harnessing biological functionality in this manner requires an interdisciplinary approach that integrates systems biology, synthetic biology, and nanofabrication. Here, we present a model system that exemplifies the synergism between these realms of research. We propose a synthetic gene network for operation in a nanofabricated cell mimic array that propagates a biomolecular signal over long distances using the phenomenon of stochastic resonance. Our system consists of a bacterial quorum sensing signal molecule, a bistable genetic switch triggered by this signal, and an array of nanofabricated cell mimic wells that contain the genetic system. An optimal level of noise in the system helps to propagate a time-varying AHL signal over long distances through the array of mimics. This noise level is determined both by the system volume and by the parameters of the genetic network. Our proposed genetically driven stochastic resonance system serves as a testbed for exploring the potential harnessing of gene expression noise to aid in the transmission of a time-varying molecular signal.

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## 1. Introduction

Given the remarkable properties of the cell [21,66] coupled with its ability to communicate and interact with other cells, it is not surprising that the idea of harnessing biological functions for engineering applications at the nanoscale has drawn much attention [52,31,23,1]. Recent years have seen the amassing of a tremendous wealth of data from the sequencing of new organisms and from high throughput expression experiments. At the same time, great progress has been made towards developing a fundamental understanding of individual cell function. The availability of well characterized biological components coupled with a deeper understanding of cell function has led to efforts both to engineer living cells and to create bio-like functionality in non-living substrates.

Living cells ranging from mammalian cells to bacteria communicate with one another using a variety of

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molecules such as calcium ions [60], peptides [43], and alcohols [15]. Here, we focus on quorum sensing systems found in Gram negative bacteria. These systems enable cells to coordinate certain functions in a population density dependent fashion [74]. In Gram negative bacteria, most quorum sensing systems are comprised of an I-protein, an R-protein, and a small signal molecule, typically an N-acyl homoserine lactone (AHL). This AHL signal can diffuse through the cell membrane into the surrounding environment. At low cell densities, only basal levels of AHL are produced. However, as local cell density increases, AHL can accumulate within the cell such that AHL binds the R-protein, changing its conformation and enabling it to bind and activate target DNA promoters. Often, one of the activated promoters controls I-protein synthesis itself, and since I-protein catalyzes AHL synthesis, a positive feedback loop is formed. Therefore, quorum sensing can allow bacteria to coordinate behavior in a density dependent manner. In nature, these quorum sensing systems have been shown to coordinate a wide range of processes such as virulence factor production [81,14], biofilm formation [19], and antimicrobial resistance [29].

Although bacterial cells can coordinate behavior at the population level, studies at the single cell level have revealed the importance of noise in gene expression. Noise is often significant due to the low number of many molecular species in the cell and due to the random and discrete nature of reactions driving cell function. Cells have evolved to either minimize or utilize this stochastic noise. In engineered systems, a great amount of work has focused on minimizing noise, especially in the field of communications. While there are some examples of using noise to improve function, such as dithering, great potential lies in the area of harnessing noise, especially in systems that operate at and below the scale of the cell.

We propose a signal propagation system that utilizes genetic components of bacterial quorum sensing systems and also harnesses noise. The signal in our system is a time-varying AHL molecule concentration. This AHL signal enters one well in an array of several nanofabricated wells. Each well contains a bistable genetic switch that can be toggled by the signal. This switch can help to amplify the signal and aid in its propagation through the array of wells. However, optimal signal propagation is realized when a particular level of noise is present in the system. This phenomenon is referred to as stochastic resonance and is further described in Section 2.

Our study demonstrates the rich potential of harnessing biological components for engineered nanocommunication networks. We also demonstrate the importance of considering the role of noise in nanoscale systems, and we show that function can actually be improved by tuning the level of noise. Furthermore, the use of nanofabricated structures enables the flexible, robust, and well-defined layout of communication channels.

Since our proposed system connects several different fields of research, we discuss background information and related work in Section 2. We describe our proposed system for stochastic resonance in Section 3 and detail materials and methods in Section 4. We present the model for our system in Section 5, and the modeling results in Section 6 demonstrate functional stochastic resonance for a variety of different conditions. In Section 7, we discuss these results and present concluding remarks.

## 2. Background and related work

Over the past decade, components of Gram negative bacterial quorum sensing systems have been employed in synthetic biology efforts to forward engineering cooperative behavior in living cells. In 2001, Weiss and Knight engineered and experimentally demonstrated "sender" and "receiver" *E. coli* cells, such as those depicted in Fig. 1(a)–(b), using components from the *V. fischeri* Lux quorum sensing system [78]. More specifically, sender cells were designed to synthesize a diffusible AHL signal, while receiver cells were engineered to express a target protein in response to AHL [78]. One advantage of utilizing Lux and other similar Gram negative quorum sensing systems is the relative simplicity of coupling these intercellular communication components to intracellular regulatory networks [77,5]. Accordingly, these as well as other components have been interfaced to synthetic gene networks for the purpose of programming cells to exhibit interesting temporal [6] and spatial [4] behaviors. For instance, they have been used to synchronize oscillations across a population of *E. coli* [46,17]. In addition, signaling specificity is sufficient [79,39,27] for at least two different AHL quorum sensing signaling pathways to operate simultaneously in the same system. This has led to the construction of synthetic consortia [13] and other engineered bacterial "ecologies" such as predator–prey systems [3].

These successful endeavors to engineer living cells are part of a broader effort to engineer biological functionality at the nanoscale. A particularly exciting frontier is nanoenabled synthetic biology which involves engineering biological behavior in non-biological substrates [21]. One challenge in this field is how to compartmentalize the molecular components that govern communication and regulation [31]. To this end several systems have used lipid vesicles such as bilayer liposomes or monolayer micelles to serve as an enclosing membrane [40,70,71,48,49,36]. Cell-free transcription and translation have been demonstrated in liposomes [47,56,82,54], and simple gene regulatory networks have been implemented [35,53].

Beyond the use of vesicles for containment of artificial cell components, another approach for containment is to create more rigid and permanent structures through nanofabrication [24,20,58]. For example, the cell mimic devices developed by Retterer et al. in Fig. 1(c) contain reaction components in small wells [58]. A microfluidic channel surrounding the wells enables the flow of reaction components through the area surrounding the well, and pores in the well walls allow the passage of components between the channel and well. Retterer et al. demonstrated enzyme reactions in these devices [58], and Siuti et al. demonstrated cell-free gene expression in similar devices [69]. These structures are particularly well suited for the construction of the theoretical communication channel which we present.

The prospect of deploying engineered cell-free gene networks in small volume nanofabricated cell mimics