Published in final edited form as:

Nano Commun Netw. 2011 March ; 2(1): 39–49. doi:10.1016/j.nancom.2011.03.001.

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

David K. Karig^a, Piro Siuti^{b,c}, Roy D. Dar^{a,d}, Scott. T. Retterer^{a,b}, Mitchel J. Doktycz^{a,b}, and Michael L. Simpson^{a,e,f,*}

^aCenter for Nanophase Materials Sciences, Oak Ridge National Laboratory, Bethel Valley Road, Oak Ridge, Tennessee 37831, USA

^bBiosciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831, USA

^cGraduate Program in Genome Science and Technology, University of Tennessee, Knoxville, Tennessee 37996, USA

^dDepartment of Physics and Astronomy, University of Tennessee, Knoxville, Tennessee 37996-2010, USA

^eDepartment of Materials Science and Engineering, University of Tennessee, Knoxville, Tennessee, USA 37996-2010, USA

^fCenter for Environmental Biotechnology, University of Tennessee, Knoxville, Tennessee 37996-2010, USA

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.

© 2011 Published by Nano communication network. All rights reserved.

*Corresponding author. Tel: +1 865 865-574-8588 Fax: +1 865 865-574-1753, karigdk@ornl.gov (D. K. Karig), psiuti@utk.edu (P. Siuti), darr@ornl.gov (R. D. Dar), rettererst@ornl.gov (S. T. Retterer), doktyczmj@ornl.gov (M. J. Doktycz), simpsonml1@ornl.gov (M. L. Simpson).

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

stochastic resonance; synthetic biology; noise; quorum sensing; cell mimic

1. Introduction

Given the remarkable properties of the cell [1, 2] coupled with its ability to communicate and interact with other cells, it is not surprising that the idea of harnessing biological function for engineering applications at the nano scale has drawn much attention [3-6]. 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 molecules such as calcium ions [7], peptides [8], and alcohols [9]. 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 [10]. 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 [11, 12], biofilm formation [13], and antimicrobial resistance [14].

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 nano communication 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 Figure 1a-b, using components from the *V. fischeri* Lux quorum sensing system [15]. 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 [15]. One advantage to utilizing Lux and other similar Gram negative quorum sensing systems is the relative simplicity of coupling these intercellular communication components to intracellular regulatory networks [16, 17]. Accordingly, these as well as other components have been interfaced to synthetic gene networks for the purpose of programming cells to exhibit interesting temporal [18] and spatial [19] behaviors. For instance, they have been used to synchronize oscillations across a population of *E. coli* [20, 21]. In addition, signaling specificity is sufficient [22-24] 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 [25] and other engineered bacterial “ecologies” such as predator-prey systems [26].

These successful endeavors to engineer living cells are part of a broader effort to engineer biological functionality at the nano scale. A particularly exciting frontier is nano-enabled synthetic biology which involves engineering biological behavior in non-biological substrates [1]. One challenge in this field is how to compartmentalize the molecular components that govern communication and regulation [4]. To this end several systems have used lipid vesicles such as bilayer liposomes or monolayer micelles to serve as an enclosing membrane [27-31] [32]. Cell-free transcription and translation have been demonstrated in liposomes [33-36], and simple gene regulatory networks have been implemented [37, 38].

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 [39-41]. For example, the cell mimic devices developed by Retterer et al in Figure 1c contain reaction components in small wells [41]. 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 passage of components between the channel and well. Retterer et al demonstrated enzyme reactions in these devices [41], and Siuti et al demonstrated cell-free gene expression in similar devices [42]. 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 offers the opportunity to both investigate and exploit functional features unique to the size and nature of living cells. Of particular interest is noise in gene

expression. Both natural and synthetic biological systems must contend with noise in gene expression [43, 44]. This is due to the small volume of cells or reaction vessels and the discrete and random nature of reactions driving function. In nature, cells minimize noise in some cases, while exploiting it in others [43]. Noise can be minimized through mechanisms such as negative feedback [45-47]. However, noise can also be harnessed in bet hedging strategies [48] and can also play a central role in functions such as the latent/lytic decision in HIV [49, 50].

Much remains to be explored in the realm of understanding how cells in nature exploit noise and likewise how engineering applications might exploit noise. As such, several modeling studies of synthetic systems have proposed interesting roles for noise in engineered biological systems. For example, Wang, Han, and Xin [51] showed that optimal magnitudes of noise (tuned by system size) can cause a system proposed by Hasty et al [52] to oscillate even when initialized in a regime of phase space just outside of the oscillatory regime. Also, Wang et al examined the role of noise in oscillation within individual cells containing a coupled genetic oscillator and bistable switch [53]. Another interesting study examined the coupled effects of cell growth, intercellular communication, and noise and how these three features can ultimately lead to reliable decisions and/or synchronous oscillations [54]. These theoretical systems refer to the concept of stochastic resonance, indicating that a particular level of noise is critical to function of the system.

Over the years, stochastic resonance has taken on a variety of different meanings [55]. However, our system is based upon the classical framework of Benzi et al [56]. This framework involves a bistable switch subjected to noise and a weak periodic signal. In the absence of noise, signal propagation is attenuated, but an optimal amount of noise enables the input to toggle the bistable switch and consequently amplify the weak signal. We investigate the plausibility of a genetically encoded version of this system, whereby a quorum sensing signal can trigger a bistable genetic switch.

3. Proposed Resonance System

The genetic components of our proposed system are the R-protein LasR, the I-protein LasI, and the *rsaL* promoter from the *P. aeruginosa* Las quorum sensing system. To demonstrate the functionality of these components in *E. coli*, we show experimental results for engineered sender and receiver cells in Figure 1a. Sender cells express the I-protein LasI, which catalyzes synthesis of the AHL signal, 3OC12HSL. This 3OC12HSL signal can diffuse into neighboring receiver cells. In the receiver cells, the R-protein LasR is constitutively expressed from the P_{laciq} promoter and can form a complex with 3OC12HSL that binds and activates the quorum sensing controlled promoter p_{qsc} , which here is the *rsaL* promoter [22, 57]. This promoter activates expression of GFP. Therefore, receiver cells closest to the senders fluoresce.

These components will be used to create a bistable genetic switch that toggles in response to AHL concentration. This genetic switch will operate in cell-free extract. In Figure 1b, we show that the receiver construct responds in cell extract. The containment platform will be based on the cell mimic devices created and demonstrated by Retterer et al [41] and Siuti et al [42] and shown in Figure 1c. We show experimental results with live *E. coli* cells in these devices in Figure 1d. Receiver cells were loaded into the cell mimic well, and M9 media with 3OC12HSL was added to the microfluidic channel. The 3OC12HSL entered the well through pores and activated GFP expression in the receiver cells.

Specifically, as shown in Figure 2, in the proposed genetic network, the quorum sensing controlled promoter expresses both R-protein and I-protein. This creates a positive feedback on signal synthesis and response. For certain parameter ranges, this positive feedback can

give rise to bistability. This cell-free genetic network is loaded into each well of the fabricated communication channel depicted in the bottom of Figure 2. Pores between the wells allow passage of small molecules such as AHL. The nanofabrication approach affords precise control over the number and size of pores, which in turn determines which components can propagate through the channel [41], meaning that pore characteristics can be chosen to restrict transport to AHL and other small molecules or to also allow flow of mRNA and even proteins. Several potential approaches can be used to fill the wells with reagents and to establish a defined flow through the communication channel. For example, the wells of the communication channel can be made individually fluidically addressable, and a pressure difference across the channel can be used to define a flow. Finally, of particular importance to our system, the well volumes can be precisely controlled. As our modeling results show, this has a critical impact on the levels of noise, which in turn plays a key role in driving stochastic resonance.

4. Materials and Methods

4.1 Live *E. coli* sender-receiver experiment

Sender cells are *E. coli* TOP10 cells transformed with the plasmid pFNK-502 [58], which expresses LasI under control of the IPTG-inducible p(lac) promoter. Receiver cells are *E. coli* TOP10 cells transformed with the plasmid pFNK-503-qscrsL [25, 58], which constitutively expresses LasR and expresses the fluorescent reporter GFP(LVA) from the quorum sensing controlled *rsaL* promoter of *Pseudomonas aeruginosa*. Sender and receiver cultures were grown overnight at 37°C in a shaking incubator. Sender cells were induced with 1 mM IPTG for 1 hour and were then concentrated approximately 50-fold. 15 μ L of the concentrated sender cell culture were dispensed onto and absorbed into a disk of blotting paper created with a standard paper hole punch. This disk was placed onto a Petri dish with 2% agarose and M9 media components previously described [25] in addition to 1mM IPTG. Small (0.2 μ L) droplets of receiver cell culture were then placed in the area surrounding the sender disk on the M9 agarose plate. The plate was then incubated at 37°C for approximately 2.5 hours prior to imaging. The mosaic image of the plate was captured on a Zeiss Axioplan 2 microscope with a Diagnostic Instruments Spot Xplorer XS Camera, a Ludl BioPrecision motorized stage, a Till Polychrome IV fluorescent excitation source, and a 10x objective. Chroma filters were used for GFP imaging with the Till excitation wavelength set at 488 nm. Images were captured using the IPLab 4.08 (Scanalytics, Inc) software package with 4 $\times$ 4 binning and a 15 ms exposure time.

4.2 Cell-free Las receiver experiment

The Promega S30 T7 High-Yield Expression System kit (Promega TM306) was used to demonstrate the cell-free functionality of the pFNK-503-qscrsL receiver plasmid. Protocol was followed according to the manufacturer's instructions for setting up the reactions, except the final reaction volume was 15 μ L, and 15 μ L mineral oil was added to prevent drying. Reactions were set up in triplicate in Corning CLS3820 plates. 3OC12HSL was purchased from Cayman. Reactions were carried out and assayed at 30°C with shaking in a Biotek Synergy 2 plate reader.

4.3 Receiver cells in cell mimic device

The transformed receiver cells were loaded in the reaction container using a glass tip with 2 μ m orifice (World Precision Instruments, TIP2TW1) and the chip was covered with a 6 mm thick layer of polydimethyl syloxane (PDMS). The channel was then filled with media containing 10 μ M AHL and fluorescence was monitored by measuring the intensity from time lapse images taken every 5 minutes for 3 hours with binning 1 $\times$ 1 and exposure time of 256 ms. A Zeiss Axioskop 2 FS Plus epifluorescent microscope equipped with a Retiga

firewire camera and a 40x dry objective (Zeiss) was used to obtain the images. The Retiga firewire camera was synchronized with a Lambda SC smart shutter (Sutter, CA) and the proper filter sets to minimize photobleaching of the fluorescent receiving cells during the experiments. The images were acquired as 16-bit grayscale TIFFs using acquisition software IPLab 4.0.8 (Scanalytics, Inc).

4.4 Simulations

All numerical work was performed using Matlab with details provided in the Section 6. The Euler-Maruyama method was used to integrate the model equations.

5. Model

For our modeling study, we assume that we have a channel consisting of an array of adjacent cell mimic wells. Each mimic well in the array contains cell extract [59] and DNA encoding the genetic network as shown in Figure 2. For simplicity and for the sake of comparison to classical non-biological stochastic resonance systems [56, 60], we model only one way propagation of the signal molecule, which corresponds to a defined flow rate through the channel. The AHL input signal flows only into the first well of the mimic channel.

Our model captures the reactions described in Table 1 including the synthesis, decay, and flow of AHL, the transcription, decay and flow of mRNA, and the translation and decay of I and R proteins. The capital letters in the first column of Table 1 indicate units of population numbers, while the corresponding lower case counterparts of these variables are concentrations. Based on these reactions in Table 1, we present a Langevin model of the system, whereby Gaussian noise terms ξ are added to ordinary differential equations to capture noise. The noise term corresponding to each reaction is also shown in Table 1, where $\eta_{j,n}(0, \sigma)$ represents independent Gaussian white noise with zero mean and standard deviation σ . Note that all noise terms ultimately scale inversely with the square root of volume (Ω).

The Langevin description of the system [61] consists of equations 1-4 below. Here a_n is the AHL concentration in the n^{th} well, m_n is the mRNA concentration in the n^{th} well, i_n is the I-protein concentration in the n^{th} well, and r_n is the R-protein concentration in the n^{th} well.

$$\frac{da_n}{dt} = k_a i_n(t) - d_a a_n(t) + c_a (a_{n-1}(t) - a_n(t)) + f_n(t) + \xi_{1,n}(t) + \xi_{2,n}(t) + \xi_{3,n-1}(t) + \xi_{3,n}(t) \quad (1)$$

$$\frac{dm_n}{dt} = p k_m \left(b + \frac{a_n(t) r_n(t)}{K + a_n(t) r_n(t)} \right) - d_m m_n(t) + c_m (m_{n-1}(t) - m_n(t)) + \xi_{4,n}(t) + \xi_{5,n}(t) + \xi_{6,n-1}(t) + \xi_{6,n}(t) \quad (2)$$

$$\frac{di_n}{dt} = k_i m_n(t) - d_i i_n(t) + \xi_{7,n}(t) + \xi_{8,n}(t) \quad (3)$$

$$\frac{dr_n}{dt} = k_r m_n(t) - d_r r_n(t) + \xi_{9,n}(t) + \xi_{10,n}(t) \quad (4)$$

Equation 1 represents reactions affecting AHL concentration in the n^{th} well. The deterministic terms of this equation, i.e. all terms other than the ξ noise terms, capture AHL synthesis catalyzed by the I-protein, AHL decay, AHL flow, and the input signal forcing term, respectively. Although any form of time-varying input signal could be used for the input signal forcing term $f_n(t)$, we define the input signal as a periodic square wave (Equation 5), corresponding to the rapid addition and cessation of AHL signal flow into the first well:

$$f_n(t) = \begin{cases} 1 + k_f \text{sgn}\left(\sin(wt) - \frac{1}{2}\right), & n=1 \\ 0, & 1 < n \leq N \end{cases} \quad (5)$$

Equation 2 models reactions affecting mRNA concentration. The first term is a Hill equation representing the rate of transcription as a function of the concentration of the AHL-R-protein complex, which scales with $a_n r_n$. The second term represents mRNA decay, while the third term represents mRNA flow between wells. Translation and decay of I-protein are represented respectively by the first two terms of Equation 3. Likewise, translation and decay of R-protein are represented respectively by the first two terms of Equation 4. Note that while AHL and mRNA can, at least for certain parameter values, flow through the wells, we do not consider cases where pore size is large enough to allow passage of either I-protein or R-protein.

The kinetic parameters used in the Langevin model are described in Table 2. We emphasize that every system parameter can be altered through genetic design, chemical means, or through device fabrication approaches. In Table 2, “pore size and number” for decay rates indicates that the device may be fabricated to allow flow of the species into an external area outside of the channel. This rate can thus be tuned by altering the pore size and number at the interface between the mimic well and the area exterior to the communication channel. In addition to the parameters shown here, c_a , c_m , and $f_n(t)$ can be controlled via the outside interface through adjustments to the flow rate and the input function.

6. Results

To quantify the fidelity of signal propagation at the n^{th} array well, we calculate a performance measure B_F based on a previously defined metric for resonant systems [60]. For a given output signal, this measure indicates how far the spectral peak at the input frequency stands above the noise background. Thus, this measure provides an indication of how well the periodicity of the input is preserved. Specifically, we examine how well AHL flow out of the n^{th} well, defined in Equation 6, preserves the periodicity of the input AHL flow $f_n(t)$. We first remove the transient component of the output signal, leaving Q samples for analysis. These Q samples are at times $t=q\Delta t$, where $q=0,1,\dots,Q-1$. We then calculate the magnitude of the Fourier transform of the output signal in Equation 7. Finally, to find the score $B_F(n)$ we subtract the noise background from the peak at the input frequency $X(w)$ as shown in Equation 8.

$$x(q\Delta t) = c_a a_n(q\Delta t) \quad (6)$$

$$X(w) = \frac{2}{Q} \left| \sum_{q=0}^{Q-1} x(q\Delta t) e^{-i w q \Delta t} \right| \quad (7)$$

$$B_F(n) = X(w) - \frac{1}{2} (X(w + \Delta w) + X(w - \Delta w)) \quad (8)$$

6.1 Demonstration of Stochastic Resonance

To initially demonstrate how noise can aid in signal propagation in our proposed system, we used the Euler-Maruyama method to integrate the set of Langevin equations (Equations 1-4) with a time step Δt of 0.5 min. Parameters were used as shown in Table 2 and as follows: $d_a = 1.26 \cdot 10^{-2} \text{ min}^{-1}$, $p = 1 \text{ nM}$, $k_i = 0.72 \text{ min}^{-1}$, $k_r = 0.72 \text{ min}^{-1}$, $c_a = c_m = 0.05 \text{ min}^{-1}$, $k_f = 5 \text{ nM/min}$, and $w = 0.001$. Figure 3a shows the AHL concentration in different wells over time in the absence of noise. The weak input signal clearly degrades with distance from the input well. Figure 3b illustrates how noise in the system can help amplify the weak input signal and aid in signal propagation. Specifically, the input signal is amplified rather than degraded, since gene network noise helps to toggle the bistable switch. Note the order of magnitude difference in y-axis scales between Figure 3a and Figure 3b. At farther array distances, the mean concentrations of AHL build. The mean can grow so high that the probability of noise causing the signal to fall below the critical threshold required for the switch to turn back off becomes exceedingly small, even during downswings in AHL. In other words, the switch tends to be stuck in the high state at far array distances. However, comparison of Figures 3a and 3b reveals that at the distances where this detrimental rise of mean AHL concentration is observed, the signal in the absence of noise has almost completely degraded.

To capture the stochastic resonance behavior over a broader range of noise levels, we then calculated scores, $B_F(n)$, for different system volumes Ω . To ensure removal of the transient component of the output signal prior to analysis, the time points before 5 input oscillations have occurred were removed. Figure 3c shows these B_F values averaged over 10 simulations of 69115 min each. These results clearly demonstrate stochastic resonance. In the absence of noise, i.e. the infinite volume case, B_F decays approximately exponentially with the array distance n in accordance with Figure 3a. Inclusion of noise results in higher B_F scores, and the optimal well volume is around $2.04 \mu\text{m}^3$. Further increasing noise by lowering the volume below this optimal value causes stronger amplification in the first few wells from the input. However, the previously mentioned buildup of mean AHL concentration also sets in earlier, thus lowering B_F scores once again.

6.2 Higher input frequencies

Having demonstrated stochastic resonance, we sought to accommodate higher input frequencies w . To do so, we examined different flow rates (c_a and c_m), different input amplitudes (k_f), and different AHL turnover rates (d_a). To show that stochastic resonance still works for these higher input frequencies, we ran Euler-Maruyama simulations using $w = 0.01$ and the following parameter changes from the Figure 3 simulations: $d_a = 7 \cdot 10^{-4} \text{ min}^{-1}$, $c_a = c_m = 0.17 \text{ min}^{-1}$, $k_f = 4.5 \text{ nM/min}$. Figure 4a captures the results of these simulations. Here, average B_F scores were calculated from 150 runs, each with a time step Δt of 0.1 min and a duration of 125664 min. To remove the transient component of the output signal prior to analysis, we removed time points before 10 input oscillations have occurred. As with the results in Figure 3, Figure 4 shows that an intermediate system volume and hence noise level is optimal for performance. Specifically, a well volume of around $6.25 \mu\text{m}^3$ appears to be optimal for long distance propagation of the time-varying information of the input signal. This volume results in the highest B_F score at the farthest distance examined ($n=99$).

6.3 Flow rate of mRNA

One useful feature of the nanofabricated platform of this system is the ability to control which species can flow from well to well based on the characteristics (e.g. size) of pores between the wells. For the results presented thus far, we assumed that mRNA can flow between wells. In Figure 4b, we show that resonance can still be observed even with no mRNA flow. The same parameters and methods were used to generate Figure 4b as Figure 4a, except that the mRNA flow rate c_m has been set to zero. The optimal system volume for long distance propagation appears to remain around $6.25 \mu\text{m}^3$.

6.4 Scaling Genetic Rates to Alter Noise Levels and accommodate larger volumes

So far, we have altered noise levels by changing the well volume. However, the parameters governing the gene network also play a large role in determining the amount of noise in the system. To show, this, we decreased p by a factor of 10 and increased k_i and k_r by a factor of 10 to enable resonance at larger volumes. It has previously been shown in simple bacterial models that decreasing the transcription rate while correspondingly increasing the translation rate can lead to bursting that increases noise [62]. In our system, as supported by Figure 4c, increasing the burst rate by decreasing the overall transcription rate while increasing the translation rate causes the optimal well volume to increase. For example, for the simulations depicted in Figure 4c, the optimal system volume is around $100 \mu\text{m}^3$.

7. Discussion

Our proposed system for exploring the use of noise to aid propagation of a time-varying AHL signal exemplifies the importance of interdisciplinary research in nanocommunication. As illustrated by Table 2, function of the overall system hinges on optimization of the nanofabricated device design, on the genetic components used, and ultimately on the noise characteristics resulting from the device and the genetic network implementations. We demonstrated functionality of the system for slower and faster input frequencies, for cases with and without flow of mRNA through the channel, and for genetic alterations designed to accommodate larger system sizes. Collectively, this shows that stochastic resonance can be harnessed for the propagation of a biological signal under a broad range of conditions.

Interestingly, for the initial set of constants examined in Figure 3 and Figure 4a-b, the optimal volumes for stochastic resonance were on the scale of bacterial cell volumes. However, we showed that larger system volumes can be accommodated by tuning the gene network to exhibit higher noise levels. Even larger system volumes could be accommodated through the employment of transcriptional bursting, which is common in eukaryotes [48, 63-65] but is also found in bacteria [66].

While molecular communication offers many advantages (small size, biocompatibility, etc.) over other communication means, two commonly cited disadvantages are speed and propagation distance [4, 6]. For the system that we proposed, speed is currently very slow. The period of the forcing function that we have used to characterize the system in Figure 4 is approximately 10 hours. However, this is not the fastest forcing frequency that the system can accommodate. Furthermore, as shown in Table 2, the system parameters can be engineered to accommodate much faster forcing frequencies. On one hand, the ability to amplify and propagate signals on this timescale is still useful. For example, it may be applicable to the amplification of circadian rhythms or other weak time varying signals in drug delivery systems. On the other hand, the system could potentially be redesigned for much faster input signals by employing faster biological machinery for implementing the switching mechanism [67]. For example, one way to make fast biological switches is to take advantage of phosphorylation/dephosphorylation cascades such as the MAPK and JNK

systems found in mammalian cells, which have been shown to exhibit bistability [68]. Another approach is to employ DNA and RNA computing methodologies, which have previously been successfully coupled to protein production machinery [69-73]. Therefore, while our system does not specifically address the issue of speed in molecular communication, it can be broadly applied to systems where issues of speed have been better resolved. Furthermore, our system does address the propagation distance aspect of molecular communication, doing so by harnessing noise in the system.

Our model assumes a forced flow through the channel and captures only one-way coupling between cell mimic wells in the array. However, one-way coupling is not a necessary condition for stochastic resonance. In general, backward propagating forces such as diffusion can hinder the performance of stochastic resonance; however, stochastic resonance can still be engineered [60]. In addition, the flow rate in these systems is generally controllable [41]. In our model, we also use a Hill equation to describe AHL/R-protein complex formation and the binding of this complex to promoter DNA. Inherent in the use of a Hill equation here is the assumption that these reactions are fast relative to other reactions in the system and that operator noise is insignificant [74].

As indicated by the scoring function results, our system preserves time varying signal information over long distances. However, the mean level of AHL can be high for distant array locations. It is likely that a wider exploration of parameter space would help to address this issue. Although Table 2 indicates that all parameters in the system can be physically tuned, we primarily focused on varying the flow rates c_a and c_m , the input amplitude k_f and the AHL decay rate d_a . Furthermore, a more complex genetic system would enable more precise tuning of the potential function of the switch to help alleviate the high mean signal concentrations at far distances. Finally, a non-uniform channel shape, i.e. one in which well volume varies with distance, could also potentially address this issue. Nonetheless, our results demonstrate that noise can be employed in a bio-inspired system to preserve information over long distances.

The precision and flexibility of the nanofabrication approach lend to scaling the system up to large numbers of compartments in a robust and repeatable fashion. One major challenge will involve loading the channels with the proper reagents and achieving a defined flow. Additionally, to scale up the system, the genetic network must be carefully tuned as discussed previously, and one area for future study is to extend and optimize the proposed genetic network to operate over even longer distances. One immediate implication of this work is that the role of noise in nanoscale systems must be carefully characterized and considered in design. Moreover, our proposed system exemplifies one of many possible ways that noise can be gainfully harnessed.

Our system shows that stochastic resonance can be implemented with genetic components in a nanocommunication system and thus illustrates the synergism between gene network design, fabrication approaches, and noise biology. While the theory of stochastic resonance was originally derived to explain periodic climate changes of the earth, the phenomenon has been applied to a number of different fields [55, 56, 75]. An interesting question is whether natural biological systems use similar stochastic resonance mechanisms to harness noise for signal propagation. For example, it could theoretically be helpful in propagating signals through colonies of bacteria or for intracellular signaling in larger eukaryotic cells. Stochastic resonance has already been implicated in circadian rhythms and in neural signaling [76-78]. Our proposed system offers a platform for investigating the fundamental aspects of these questions.

Acknowledgments

The Las sender and receiver plasmids used in the Figure 1 experiments were constructed in Dr. Ron Weiss's lab and were used with his kind permission. P.S. and M.J.D. acknowledge support from NIH Grant EB000657. D.K.K., R.D.D., S.T.R. and M.L.S. were supported by the in-house research program of the Center for Nanophase Materials Sciences that is sponsored by the Scientific User Facilities Division, U.S. Department of Energy. This work was performed at Oak Ridge National Laboratory.

Brief Biography

David K. Karig is a Postdoctoral Research Associate in the Center for Nanophase Materials Science (CNMS) at Oak Ridge National Laboratory (ORNL). He earned his B.S. in Electrical Engineering from Clemson University in 2000 and his Ph.D. in Electrical Engineering from Princeton University in 2007. David's research is in the fields of synthetic and systems biology, with particular interests in noise, pattern formation, and quorum sensing.

Piro Siuti received his B.S. in Biology with a minor in Chemistry from University of Southern Indiana in 2005. He is working toward his doctorate from the Graduate School of Genome Science and Technology (GST) at the University of Tennessee under the direction of Dr. Mitchel J. Doktycz. Piro's research is in the field of synthetic biology, with particular interests in the use of nanostructures to study and mimic biological systems, enzyme reactions and microbial interactions.

Roy D. Dar received his B.Sc. in Physics and Mathematics from the Hebrew University of Jerusalem, Israel (HUJI, 2004). His doctorate in Biological Physics at the University of Tennessee under the direction of Dr. Michael Simpson is expected in 2011. Roy's dissertation is on the "Adaptation and Stochasticity of Natural Complex Systems" and falls under the "Emergent Behaviors" theme at Oak Ridge National Laboratory's (ORNL's) Center for Nanophase Materials Sciences (CNMS). Roy's interests include using stochastic gene expression to probe gene circuit structure-function relationships and has worked with both synthetic and natural gene circuits in *E.coli*, yeast, and mammalian cells.

Scott T. Retterer is a research scientist in the Biological and Nanoscale Systems Group (BNSG) and shares an appointment in the Center for Nanophase Materials Science (CNMS) at Oak Ridge National Laboratory (ORNL). Scott received his B.S. in Mechanical Engineering from the University of Illinois at Chicago in 2000 and completed his Ph.D. in Biomedical Engineering at Cornell University, Ithaca, NY in 2005. Scott was a postdoctoral research fellow in the BNSG laboratory from 2005-2006 before being appointed to a joint staff position in the Biosciences and CNMS divisions. Scott currently works on a variety of projects that connect state of the art nanofabrication and characterization techniques with fundamental biomedical research.

Mitchel J. Doktycz is a Distinguished Staff Scientist and Leader of the Biological and Nanoscale Systems Group in the Biosciences Division at the Oak Ridge National Laboratory. He received his B.S. in Biology and Chemistry in 1985 and Ph.D. in Chemistry in 1991 from the University of Illinois at Chicago. His research interests focus at the intersection of biological and nanoscale systems. His laboratory is involved in the development of analytical technologies for post-genomics studies with specific emphases on molecular and cellular imaging techniques and the use of nanomaterials to study and mimic biological systems.

Michael L. Simpson is a Distinguished R&D Staff Member and the Group Leader of the Nanofabrication Research Laboratory in the Center for Nanophase Materials Sciences. In addition, he is a Professor of Material Science and Engineering at the University of

Tennessee, Knoxville (UTK). Simpson received a PhD in Electrical Engineering from UTK in 1991 and joined the staff of the Oak Ridge National Laboratory in 1992. He made key contributions to the introduction of custom integrated circuits to medium and high energy physics instrumentation before moving his research to the interfaces of engineering, physical sciences, and biology. In this arena Simpson made important contributions to the use of nanotechnology to enable the transfer of information across the biological-synthetic device boundary and the development of a fundamental understanding of Noise Biology. Simpson is a Fellow of the IEEE and the AIMBE, and a Battelle Memorial Institute Distinguished Inventor, and he serves on the editorial boards of the Journal of Nanomedicine and Biotherapeutic Discovery and the International Journal of Natural Computing Research.

Abbreviations

AHL	N-acylhomoserine lactone
IPTG	Isopropyl β -D-1-thiogalactopyranoside
3OC12HSL	3-oxo-C12-homoserine lactone
GFP	green fluorescent protein

References

- [1]. Doktycz MJ, Simpson ML. Nano-enabled synthetic biology. *Mol Syst Biol.* 2007; 3:125. [PubMed: 17625513]
- [2]. Simpson ML, Cox CD, Peterson GD, Saylor GS. Engineering in the biological substrate: information processing in genetic circuits. *Proceedings of the IEEE.* 2004; 92:848–863.
- [3]. Nakano T, Shuai J, Koujin T, Suda T, Hiraoka Y, Haraguchi T. Biological excitable media based on non-excitable cells and calcium signaling. *Nano Communication Networks.* 2010; 1:43–49.
- [4]. Hiyama S, Moritani Y. Molecular communication: Harnessing biochemical materials to engineer biomimetic communication systems. *Nano Communication Networks.* 2010; 1:20–30.
- [5]. Fernandes R, Roy V, Wu H-C, Bentley WE. Engineered biological nanofactories trigger quorum sensing response in targeted bacteria. *Nat Nano.* 2010; 5:213–217.
- [6]. Akyildiz IF, Brunetti F, Blázquez C. Nanonetworks: A new communication paradigm. *Computer Networks.* 2008; 52:2260–2279.
- [7]. Sanderson MJ, Charles AC, Boitano S, Dirksen ER. Mechanisms and function of intercellular calcium signaling. *Molecular and Cellular Endocrinology.* 1994; 98:173–187. [PubMed: 8143927]
- [8]. Lyon GJ, Novick RP. Peptide signaling in *Staphylococcus aureus* and other Gram-positive bacteria. *Peptides.* 2004; 25:1389–1403. [PubMed: 15374643]
- [9]. Chen H, Fink GR. Feedback control of morphogenesis in fungi by aromatic alcohols. *Genes Dev.* 2006; 20:1150–1161. [PubMed: 16618799]

- [10]. Waters CM, Bassler BL. QUORUM SENSING: Cell-to-Cell Communication in Bacteria. Annual Review of Cell and Developmental Biology. 2005; 21:319–346.
- [11]. Winzer K, Falconer C, Garber NC, Diggle SP, Camara M, Williams P. The *Pseudomonas aeruginosa* lectins PA-IL and PA-IIL are controlled by quorum sensing and by RpoS. J Bacteriol. 2000; 182:6401–6411. [PubMed: 11053384]
- [12]. Brint JM, Ohman DE. Synthesis of multiple exoproducts in *Pseudomonas aeruginosa* is under the control of RhlR-RhlI, another set of regulators in strain PAO1 with homology to the autoinducer-responsive LuxR-LuxI family. J Bacteriol. 1995; 177:7155–7163. [PubMed: 8522523]
- [13]. Davies DG, Parsek MR, Pearson JP, Iglewski BH, Costerton JW, Greenberg EP. The involvement of cell-to-cell signals in the development of a bacterial biofilm. Science. 1998; 280:295–298. [PubMed: 9535661]
- [14]. Hassett DJ, Ma JF, Elkins JG, McDermott TR, Ochsner UA, West SE, Huang CT, Fredericks J, Burnett S, Stewart PS, McFeters G, Passador L, Iglewski BH. Quorum sensing in *Pseudomonas aeruginosa* controls expression of catalase and superoxide dismutase genes and mediates biofilm susceptibility to hydrogen peroxide. Mol Microbiol. 1999; 34:1082–1093. [PubMed: 10594832]
- [15]. Weiss R.; Knight, T. DNA Computing. Springer; Berlin / Heidelberg: 2001. Engineered communications for microbial robotics; p. 1-16.
- [16]. Weiss R, Basu S, Hooshangi S, Kalmbach A, Karig D, Mehreja R, Netravali I. Genetic circuit building blocks for cellular computation, communications, and signal processing. Natural Computing. 2003; 2:47–84.
- [17]. Basu S, Karig D, Weiss R. Engineering signal processing in cells: Towards molecular concentration band detection. Natural Computing. 2003; 2:463–478.
- [18]. Basu S, Mehreja R, Thiberge S, Chen M-T, Weiss R. Spatiotemporal control of gene expression with pulse-generating networks. Proceedings of the National Academy of Sciences of the United States of America. 2004; 101:6355–6360. [PubMed: 15096621]
- [19]. Basu S, Gerchman Y, Collins CH, Arnold FH, Weiss R. A synthetic multicellular system for programmed pattern formation. Nature. 2005; 434:1130–1134. [PubMed: 15858574]
- [20]. McMillen D, Kopell N, Hasty J, Collins JJ. Synchronizing genetic relaxation oscillators by intercell signaling. Proceedings of the National Academy of Sciences of the United States of America. 2002; 99:679–684. [PubMed: 11805323]
- [21]. Danino T, Mondragon-Palomino O, Tsimring L, Hasty J. A synchronized quorum of genetic clocks. Nature. 2010; 463:326–330. [PubMed: 20090747]
- [22]. Whiteley M, Greenberg EP. Promoter specificity elements in *Pseudomonas aeruginosa* quorum-sensing-controlled genes. J Bacteriol. 2001; 183:5529–5534. [PubMed: 11544214]
- [23]. Karig D, Weiss R. Signal-amplifying genetic circuit enables in vivo observation of weak promoter activation in the Rhl quorum sensing system. Biotechnol Bioeng. 2005; 89:709–718. [PubMed: 15641095]
- [24]. Gray KM, Passador L, Iglewski BH, Greenberg EP. Interchangeability and specificity of components from the quorum-sensing regulatory systems of *Vibrio fischeri* and *Pseudomonas aeruginosa*. J Bacteriol. 1994; 176:3076–3080. [PubMed: 8188610]
- [25]. Brenner K, Karig DK, Weiss R, Arnold FH. Engineered bidirectional communication mediates a consensus in a microbial biofilm consortium. Proc Natl Acad Sci U S A. 2007; 104:17300–17304. [PubMed: 17959781]
- [26]. Balagadde FK, Song H, Ozaki J, Collins CH, Barnet M, Arnold FH, Quake SR, You L. A synthetic *Escherichia coli* predator-prey ecosystem. Mol Syst Biol. 2008; 4:187. [PubMed: 18414488]
- [27]. Karlsson M, Davidson M, Karlsson R, Karlsson A, Bergenholtz J, Konkoli Z, Jesorka A, Lobovkina T, Hurtig J, Voinova M, Orwar O. BIOMIMETIC NANOSCALE REACTORS AND NETWORKS. Annual Review of Physical Chemistry. 2004; 55:613–649.
- [28]. Vriezema DM, Comellas Aragon A, Elemans JAAW, Cornelissen JJLM, Rowan AE, Nolte RJM. Self-Assembled Nanoreactors. Chemical Reviews. 2005; 105:1445–1490. [PubMed: 15826017]
- [29]. Walde P, Ichikawa S. Enzymes inside lipid vesicles: preparation, reactivity and applications. Biomolecular Engineering. 2001; 18:143–177. [PubMed: 11576871]

- [30]. Moritani, Y.; Hiyama, S.; Nomura, SM.; Akiyoshi, K.; Suda, T. A Communication interface using vesicles embedded with channel forming proteins in molecular communication; Bio-Inspired Models of Network, Information and Computing Systems; 2007; 2007. p. 147-149. *Bionetics* 2007. 2nd
- [31]. Moritani, Y.; Hiyama, S.; Suda, T. Molecular communication among nanomachines using vesicles; *NSTI-Nanotech* 2006; 2006;
- [32]. Jesorka A, Orwar O. Liposomes: technologies and analytical applications. *Annu Rev Anal Chem* (Palo Alto Calif). 2008; 1:801–832. [PubMed: 20636098]
- [33]. Monnard PA. Liposome-entrapped Polymerases as Models for Microscale/Nanoscale Bioreactors. *Journal of Membrane Biology*. 2003; 191:87–97. [PubMed: 12533776]
- [34]. Pereira de Souza T, Stano P, Luisi PL. The minimal size of liposome-based model cells brings about a remarkably enhanced entrapment and protein synthesis. *Chembiochem*. 2009; 10:1056–1063. [PubMed: 19263449]
- [35]. Yu W, Sato K, Wakabayashi M, Nakaishi T, Ko-Mitamura EP, Shima Y, Urabe I, Yomo T. Synthesis of functional protein in liposome. *J Biosci Bioeng*. 2001; 92:590–593. [PubMed: 16233152]
- [36]. Nomura SM, Tsumoto K, Hamada T, Akiyoshi K, Nakatani Y, Yoshikawa K. Gene expression within cell-sized lipid vesicles. *Chembiochem*. 2003; 4:1172–1175. [PubMed: 14613108]
- [37]. Ishikawa K, Sato K, Shima Y, Urabe I, Yomo T. Expression of a cascading genetic network within liposomes. *FEBS Lett*. 2004; 576:387–390. [PubMed: 15498568]
- [38]. Noireaux V, Bar-Ziv R, Godefroy J, Salman H, Libchaber A. Toward an artificial cell based on gene expression in vesicles. *Phys Biol*. 2005; 2:P1–8. [PubMed: 16224117]
- [39]. Fletcher BL, Hullander ED, Melechko AV, McKnight TE, Klein KL, Hensley DK, Morrell JL, Simpson ML, Doktycz MJ. Microarrays of Biomimetic Cells Formed by the Controlled Synthesis of Carbon Nanofiber Membranes. *Nano Letters*. 2004; 4:1809–1814.
- [40]. Doktycz, MJ.; Fletcher, BL.; Hullander, ED.; Fowlkes, JD.; Retterer, ST.; McKnight, TE.; Melechko, AV.; Simpson, ML. Cell mimics created from the controlled synthesis and directed assembly of carbon nanofibers; *Bio Micro and Nanosystems Conference*, 2006. BMN '06; 2006; p. 13-13.
- [41]. Retterer ST, Siuti P, Choi C-K, Thomas DK, Doktycz MJ. Development and fabrication of nanoporous silicon-based bioreactors within a microfluidic chip. *Lab on a Chip*. 2010; 10:1174–1181. [PubMed: 20390137]
- [42]. Siuti, P.; Retterer, ST.; Chang Kyoung, C.; Fowlkes, JD.; Doktycz, MJ. Cell free translation in engineered picoliter volume containers; *Biomedical Science & Engineering Conference*, 2009. BSEC 2009. First Annual ORNL; 2009; p. 1-4.
- [43]. Simpson ML, Cox CD, Allen MS, McCollum JM, Dar RD, Karig DK, Cooke JF. Noise in biological circuits. *Wiley Interdiscip Rev Nanomed Nanobiotechnol*. 2009; 1:214–225. [PubMed: 20049792]
- [44]. Bewick S, Yang R, Zhang M. Complex mathematical models of biology at the nanoscale. *Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology*. 2009; 1:650–659. [PubMed: 20049822]
- [45]. Simpson ML, Cox CD, Sayler GS. Frequency domain analysis of noise in autoregulated gene circuits. *Proc Natl Acad Sci U S A*. 2003; 100:4551–4556. [PubMed: 12671069]
- [46]. Becskei A, Serrano L. Engineering stability in gene networks by autoregulation. *Nature*. 2000; 405:590–593. [PubMed: 10850721]
- [47]. Austin DW, Allen MS, McCollum JM, Dar RD, Wilgus JR, Sayler GS, Samatova NF, Cox CD, Simpson ML. Gene network shaping of inherent noise spectra. *Nature*. 2006; 439:608–611. [PubMed: 16452980]
- [48]. Blake WJ, Balazsi G, Kohanski MA, Isaacs FJ, Murphy KF, Kuang Y, Cantor CR, Walt DR, Collins JJ. Phenotypic consequences of promoter-mediated transcriptional noise. *Mol Cell*. 2006; 24:853–865. [PubMed: 17189188]
- [49]. Weinberger LS, Dar RD, Simpson ML. Transient-mediated fate determination in a transcriptional circuit of HIV. *Nat Genet*. 2008; 40:466–470. [PubMed: 18344999]

- [50]. Weinberger LS, Burnett JC, Toettcher JE, Arkin AP, Schaffer DV. Stochastic gene expression in a lentiviral positive-feedback loop: HIV-1 Tat fluctuations drive phenotypic diversity. *Cell*. 2005; 122:169–182. [PubMed: 16051143]
- [51]. Wang Z, Hou Z, Xin H. Internal noise stochastic resonance of synthetic gene network. *Chemical Physics Letters*. 2005; 401:307–311.
- [52]. Hasty J, Dolnik M, Rottsch, auml, fer V, Collins JJ. Synthetic Gene Network for Entraining and Amplifying Cellular Oscillations. *Physical Review Letters*. 2002; 88:148101. [PubMed: 11955179]
- [53]. Wang Z, Hou Z, Xin H, Zhang Z. Engineered internal noise stochastic resonator in gene network: A model study. *Biophysical Chemistry*. 2007; 125:281–285. [PubMed: 17081673]
- [54]. Koseska A, Zaikin A, Kurths J, Garcia-Ojalvo J. Timing Cellular Decision Making Under Noise via Cell-Cell Communication. *PLoS ONE*. 2009; 4:e4872. [PubMed: 19283068]
- [55]. McDonnell MD, Abbott D. What is stochastic resonance? Definitions, misconceptions, debates, and its relevance to biology. *PLoS Comput Biol*. 2009; 5:e1000348. [PubMed: 19562010]
- [56]. Benzi R, et al. The mechanism of stochastic resonance. *Journal of Physics A: Mathematical and General*. 1981; 14:L453.
- [57]. Schuster M, Urbanowski ML, Greenberg EP. Promoter specificity in *Pseudomonas aeruginosa* quorum sensing revealed by DNA binding of purified LasR. *Proceedings of the National Academy of Sciences of the United States of America*. 2004; 101:15833–15839. [PubMed: 15505212]
- [58]. Karig, D. Ph.D. thesis. Princeton University; 2007. Engineering multi-signal synthetic biological systems.
- [59]. Shin J, Noireaux V. Efficient cell-free expression with the endogenous *E. Coli* RNA polymerase and sigma factor 70. *Journal of Biological Engineering*. 2010; 4:8. [PubMed: 20576148]
- [60]. Zhang Y, Hu G, Gammaioni L. Signal transmission in one-way coupled bistable systems: Noise effect. *Physical Review E*. 1998; 58:2952.
- [61]. Gillespie DT. The chemical Langevin equation. *The Journal of Chemical Physics*. 2000; 113:297–306.
- [62]. Ozbudak EM, Thattai M, Kurtser I, Grossman AD, van Oudenaarden A. Regulation of noise in the expression of a single gene. *Nat Genet*. 2002; 31:69–73. [PubMed: 11967532]
- [63]. Raj A, van Oudenaarden A. Nature, nurture, or chance: stochastic gene expression and its consequences. *Cell*. 2008; 135:216–226. [PubMed: 18957198]
- [64]. Murphy KF, Adams RM, Wang X, Balazsi G, Collins JJ. Tuning and controlling gene expression noise in synthetic gene networks. *Nucleic Acids Res*. 2010; 38:2712–2726. [PubMed: 20211838]
- [65]. Dar RD, Karig DK, Cooke JF, Cox CD, Simpson ML. Distribution and regulation of stochasticity and plasticity in *Saccharomyces cerevisiae*. *Chaos: An Interdisciplinary Journal of Nonlinear Science*. 2010; 20:037106.
- [66]. Choi PJ, Cai L, Frieda K, Xie XS. A Stochastic Single-Molecule Event Triggers Phenotype Switching of a Bacterial Cell. *Science*. 2008; 322:442–446. [PubMed: 18927393]
- [67]. Bialek, W. Stability and Noise in Biochemical Switches. In: Leen, T.; Dietterich, T.; Tresp, V., editors. *Advances in Neural Information Processing*. Vol. 13. MIT Press; Cambridge: 2001.
- [68]. Markevich NI, Hoek JB, Kholodenko BN. Signaling switches and bistability arising from multisite phosphorylation in protein kinase cascades. *J Cell Biol*. 2004; 164:353–359. [PubMed: 14744999]
- [69]. Win MN, Smolke CD. Higher-Order Cellular Information Processing with Synthetic RNA Devices. *Science*. 2008; 322:456–460. [PubMed: 18927397]
- [70]. Simpson ML. Cell-free synthetic biology: a bottom-up approach to discovery by design. *Mol Syst Biol*. 2006; 2
- [71]. Rinaudo K, Bleris L, Maddamsetti R, Subramanian S, Weiss R, Benenson Y. A universal RNAi-based logic evaluator that operates in mammalian cells. *Nat Biotech*. 2007; 25:795–801.
- [72]. Kim J, White KS, Winfree E. Construction of an in vitro bistable circuit from synthetic transcriptional switches. *Mol Syst Biol*. 2006; 2:68. [PubMed: 17170763]

- [73]. Karig DK, Simpson ML. Tying new knots in synthetic biology. *HFSP J.* 2008; 2:124–128. [PubMed: 19404464]
- [74]. Simpson ML, Cox CD, Saylor GS. Frequency domain chemical Langevin analysis of stochasticity in gene transcriptional regulation. *Journal of Theoretical Biology.* 2004; 229:383–394. [PubMed: 15234205]
- [75]. Benzi R, Parisi G, Sutera A, Vulpiani A. A Theory of Stochastic Resonance in Climatic Change. *SIAM Journal on Applied Mathematics.* 1983; 43:565–578.
- [76]. Moss F, Ward LM, Sannita WG. Stochastic resonance and sensory information processing: a tutorial and review of application. *Clin Neurophysiol.* 2004; 115:267–281. [PubMed: 14744566]
- [77]. Hou Z, Xin H. Internal noise stochastic resonance in a circadian clock system. *The Journal of Chemical Physics.* 2003; 119:11508–11512.
- [78]. Hanggi P. Stochastic resonance in biology. How noise can enhance detection of weak signals and help improve biological information processing. *Chemphyschem.* 2002; 3:285–290. [PubMed: 12503175]
- [79]. Huang JJ, Petersen A, Whiteley M, Leadbetter JR. Identification of QuiP, the Product of Gene PA1032, as the Second Acyl-Homoserine Lactone Acylase of *Pseudomonas aeruginosa* PAO1. *Appl. Environ. Microbiol.* 2006; 72:1190–1197. [PubMed: 16461666]
- [80]. Huang JJ, Han J-I, Zhang L-H, Leadbetter JR. Utilization of Acyl-Homoserine Lactone Quorum Signals for Growth by a Soil *Pseudomonad* and *Pseudomonas aeruginosa* PAO1. *Appl. Environ. Microbiol.* 2003; 69:5941–5949. [PubMed: 14532048]
- [81]. Dong Y-H, Wang L-H, Xu J-L, Zhang H-B, Zhang X-F, Zhang L-H. Quenching quorum-sensing-dependent bacterial infection by an N-acyl homoserine lactonase. *Nature.* 2001; 411:813–817. [PubMed: 11459062]
- [82]. Shin J, Noireaux V. Study of messenger RNA inactivation and protein degradation in an *Escherichia coli* cell-free expression system. *J Biol Eng.* 2010; 4:9. [PubMed: 20594314]
- [83]. Zhang Y, Zhang J, Hara H, Kato I, Inouye M. Insights into the mRNA cleavage mechanism by MazF, an mRNA interferase. *J Biol Chem.* 2005; 280:3143–3150. [PubMed: 15537630]
- [84]. Gottesman S, Roche E, Zhou Y, Sauer RT. The ClpXP and ClpAP proteases degrade proteins with carboxy-terminal peptide tails added by the SsrA-tagging system. *Genes & Development.* 1998; 12:1338–1347. [PubMed: 9573050]

Fig. 1.

Platform components for proposed system. (a) Top left: *E. coli* cells harboring sender constructs express the I-protein (LasI here), which catalyzes synthesis of the AHL signal 3OC12HSL. Top right: *E. coli* cells harboring receiver constructs express the R-protein (LasR here) which forms a complex with the AHL signal that binds and activates a quorum sensing promoter p_{qsc} (the *rsaL* promoter here). Bottom: Experimental results of a disk of sender *E. coli* cells activating nearby droplets of receiver cells. (b) Receiver cells function in cell-free extract (c) Cell mimic devices

Fig. 2.

Proposed system for harnessing stochastic resonance. Top: Bistable genetic switch expressing both R-protein and I-protein from the quorum sensing activated p_{qsc} promoter. Bottom left: AHL flows into the first well of the communication channel in a time-varying fashion according to the function $f(t)$. Bottom right: The nanofabricated communication channel is composed of individual small wells, each of which contains cell extract and the genetic network. Pores between the wells allow flow of small molecules such as the AHL signal.

Fig. 3.

Demonstration of stochastic resonance. (a) AHL concentration vs. time for different array wells with no noise in the system. (b) AHL concentration vs. time for different array wells with an optimal level of noise in the system. (c) $B_F(n)$ scores for different well volumes (μm^3).

Fig. 4. Simulations demonstrating stochastic resonance with a faster input frequency $w=0.01$ and a) with flow of mRNA b) without flow of mRNA and c) with p decreased by a factor of 10 and k_i and k_r increased by a factor of 10 to enable resonance at larger volumes (μm^3).

Table 1

Reaction	Description	Reaction Rate	Langevin Noise Term $\xi_{j,n}(t)$
$I_n \rightarrow I_n + A_n$	AHL synthesis	$k_a(i_n\Omega)$	$\xi_{1,n}(t) = \eta_{1,n}(0, (k_a i_n / \Omega)^{1/2})$
$A_n \rightarrow *$	AHL decay	$d_a(i_n\Omega)$	$\xi_{2,n}(t) = \eta_{2,n}(0, (d_a i_n / \Omega)^{1/2})$
$A_{n-1} \rightarrow A_n$	AHL flow into well	$c_a(a_{n-1}\Omega)$	$\xi_{3,n-1}(t) = \eta_{3,n}(0, (c_a a_{n-1} / \Omega)^{1/2})$
$P \rightarrow M_n$	Transcription	$k_m(p\Omega)(b + a_n r_n / (K + a_n r_n))$	$\xi_{4,n}(t) = \eta_{4,n}(0, (k_m p / \Omega)^{1/2} (b + a_n r_n / (K + a_n r_n))^{1/2})$
$M_n \rightarrow *$	mRNA decay	$d_m(m_n\Omega)$	$\xi_{5,n}(t) = \eta_{5,n}(0, (d_m m_n / \Omega)^{1/2})$
$M_{n-1} \rightarrow M_n$	mRNA flow into well	$c_m(m_{n-1}\Omega)$	$\xi_{6,n-1}(t) = \eta_{6,n}(0, (c_m m_{n-1} / \Omega)^{1/2})$
$M_n \rightarrow M_n + I_n$	I translation	$k_i(m_n\Omega)$	$\xi_{7,n}(t) = \eta_{7,n}(0, (k_i m_n / \Omega)^{1/2})$
$I_n \rightarrow *$	I decay	$d_i(i_n\Omega)$	$\xi_{8,n}(t) = \eta_{8,n}(0, (d_i i_n / \Omega)^{1/2})$
$M_n \rightarrow M_n + R_n$	R translation	$k_r(m_n\Omega)$	$\xi_{9,n}(t) = \eta_{9,n}(0, (k_r m_n / \Omega)^{1/2})$
$R_n \rightarrow *$	R decay	$d_r(r_n\Omega)$	$\xi_{10,n}(t) = \eta_{10,n}(0, (d_r r_n / \Omega)^{1/2})$

Table 2

Parameter	Description	Value(s) used	Means of altering
Ω	Volume of single mimic well	1-1000 μm^3	Fabrication of different device sizes
k_a	AHL synthesis rate	0.1 min^{-1}	Temperature, precursor concentrations
d_a	AHL decay rate	$7 \cdot 10^{-4} - 1.26 \cdot 10^{-2} \text{ min}^{-1}$	Acylase[79, 80], lactonase[81], pH, pore size and number
p	Promoter copy number	1 nM	Adding different DNA concentrations
k_m	Maximum transcription rate	1 min^{-1}	Promoter variants/mutations [57]
b	Basal expression	0.00286	Promoter variants/mutations [57]
K	Concentration of AHL/R-protein complex required for half maximal promoter expression	60000 nM ²	Promoter variants/mutations [57]
d_m	mRNA decay rate	0.321 min^{-1}	MaxEF mRNA cleavage [82, 83]; pore size and number
k_i	Translation rate of I-protein	0.72-7.2 min^{-1}	Ribosome binding site mutations
d_i	Decay rate of I-protein	0.0116 min^{-1}	ssrA protease tag [84]; pore size and number
k_r	Translation rate of R-protein	0.72-7.2 min^{-1}	Ribosome binding site mutations
d_r	Decay rate of R-protein	0.0116 min^{-1}	ssrA protease tag [84]; pore size and number